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2. Patent application number (The Patent Office will fill this part) — 0404567.0 — 1 MAR 2004

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

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Patents ADP number *(if you know it)* 8584922001

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4. Title of the invention — METHODS FOR TREATING BLADDER DYSFUNCTION AND RELATED COMPOUNDS AND COMPOSITIONS

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(2) PF 51/77 18/5/04
(3) 51/77 14/9/04
(4) 51/77 16/2/05

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Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

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Abstract

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Priority documents

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11. I/We request the grant of a patent on the basis of this application.

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Date 1 March 2004

12. Name and daytime telephone number of person to contact in the United Kingdom

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METHODS FOR TREATING BLADDER DYSFUNCTION AND RELATED COMPOUNDS AND COMPOSITIONS

The present invention is concerned with the use of vitamin D compounds for the manufacture of a medicament for the prevention and/or treatment of bladder dysfunction, especially dysfunction related to morphological bladder changes. It is further concerned with a method for preventing and/or treating bladder dysfunction, especially dysfunction related to morphological bladder changes, by administering a vitamin D compound in an amount effective to prevent and/or to treat such disease alone or in combination with further agents.

Morphological bladder changes, including a progressive de-nervation and hypertrophy of the bladder wall are frequent histological findings in patients with different bladder disorders such as overactive bladder and clinical BPH.

The increase in tension and/or strain on the bladder observed in these conditions has been shown to be associated with cellular and molecular alterations, e.g., in cytoskeletal and contractile proteins, in mitochondrial function, and in various enzyme activities of the smooth muscle cells. The growth of the bladder wall also involves alterations in its extracellular matrix and non-smooth muscle components.

These changes in the bladder are associated with the storage (irritative) symptoms, in particular frequency, urgency and nocturia. These symptoms affect the social, psychological, domestic, occupational, physical and sexual lives of the patients leading to a profound, negative impact on their quality of life.

At the present time, an ideal treatment of these symptoms has not been found. Each of the therapeutic options available (for example, anti-muscarinics, alpha-blockers) is associated with disadvantages relating to their mechanism of action, which is based only on the management of symptoms.

and not on the treatment of the etiology of the condition. In fact, the clinical utility of some of the available agents has been limited by poor patient acceptance and compliance.

As a consequence there is a need for new treatments that provide improved clinical effectiveness by targeting the underlying etiological factor , the abnormal growth and consequent decompensation of bladder smooth muscle cells.

As described herein, it has now surprisingly been found that vitamin D analogues can treat and prevent bladder dysfunction, such as disorders associated with bladder hypertrophy, such as bladder overactivity and clinical BPH.

In addition, such vitamin D related compounds have an application in the treatment of storage (irritative) symptoms of benign prostatic hyperplasia (BPH). BPH is associated not only with enlargement of the gland leading to bladder outlet obstruction (BOO) and symptoms secondary to this, but also to morphological bladder changes, including a progressive denervation and hypertrophy of the bladder wall, the latter possibly as a consequence of increased functional demands.

The importance of vitamin D (cholecalciferol) in the biological systems of higher animals has been recognized since its discovery by Mellanby in 1920 (Mellanby, E. (1921) Spec. Rep. Ser. Med. Res. Council (GB) SRS 61:4). It was in the interval of 1920-1930 that vitamin D officially became classified as a "vitamin" that was essential for the normal development of the skeleton and maintenance of calcium and phosphorous homeostasis.

Studies involving the metabolism of vitamin D_3 were initiated with the discovery and chemical characterization of the plasma metabolite, 25-hydroxyvitamin D_3 [25(OH)D_3] (Blunt, J.W. et al. (1968) Biochemistry 6:3317-3322) and the hormonally active form, 1α,25$(OH)_2D_3$ (Myrtle, J.F. et al. (1970) J. Biol. Chem. 245:1190-1196; Norman, A.W. et al. (1971) Science 173:51-

54; Lawson, D.E.M. et al. (1971) Nature 230:228-230; Holick, M.F. (1971) Proc. Natl. Acad. Sci. USA 68:803-804). The formulation of the concept of a vitamin D endocrine system was dependent both upon appreciation of the key role of the kidney in producing 1α, 25$(OH)_2D_3$ in a carefully regulated fashion (Fraser, D.R. and Kodicek, E (1970) Nature 288:764-766; Wong, R.G. et al. (1972) J. Clin. Invest. 51:1287-1291), and the discovery of a nuclear receptor for 1α,25$(OH)_2D_3$ (VD_3R) in the intestine (Haussler, M.R. et al. (1969) Exp. Cell Res. 58:234-242; Tsai, H.C. and Norman, A.W. (1972) J. Biol. Chem. 248:5967-5975).

The operation of the vitamin D endocrine system depends on the following: first, on the presence of cytochrome P450 enzymes in the liver (Bergman, T. and Postlind, H. (1991) Biochem. J. 276:427-432; Ohyama, Y and Okuda, K. (1991) J. Biol. Chem. 266:8690-8695) and kidney (Henry, H.L. and Norman, A.W. (1974) J. Biol. Chem. 249:7529-7535; Gray, R.W. and Ghazarian, J.G. (1989) Biochem. J. 259:561-568), and in a variety of other tissues to effect the conversion of vitamin D_3 into biologically active metabolites such as 1α, 25$(OH)_2D_3$ and 24R,25$(OH)_2D_3$; second, on the existence of the plasma vitamin D binding protein (DBP) to effect the selective transport and delivery of these hydrophobic molecules to the various tissue components of the vitamin D endocrine system (Van Baelen, H. et al. (1988) Ann NY Acad. Sci. 538:60-68; Cooke, N.E. and Haddad, J.G. (1989) Endocr. Rev. 10:294-307; Bikle, D.D. et al. (1986) J. Clin. Endocrinol. Metab. 63:954-959); and third, upon the existence of stereoselective receptors in a wide variety of target tissues that interact with the agonist 1α,25$(OH)_2D_3$ to generate the requisite specific biological responses for this secosteroid hormone (Pike, J.W. (1991) Annu. Rev. Nutr. 11:189-216). To date, there is evidence that nuclear receptors for 1α,25$(OH)_2D_3$ (VD_3R) exist in more than 30 tissues and cancer cell lines (Reichel, H. and Norman, A.W. (1989) Annu. Rev. Med. 40:71-78), including the normal bladder.

Vitamin D_3 and its hormonally active forms are well-known regulators of calcium and phosphorous homeostasis. These compounds are known to stimulate, at least one of, intestinal absorption of calcium and phosphate, mobilization of bone mineral, and retention of calcium in the kidneys. Furthermore, the discovery of the presence of specific vitamin D receptors in more than 30 tissues has led to the identification of vitamin D_3 as a pluripotent regulator outside its classical role in calcium/bone homeostasis. A paracrine role for $1\alpha,25(OH)_2$ D_3 has been suggested by the combined presence of enzymes capable of oxidizing vitamin D_3 into its active forms, e.g., 25-OHD-1α -hydroxylase, and specific receptors in several tissues such as bone, keratinocytes, placenta, and immune cells. Moreover, vitamin D_3 hormone and active metabolites have been found to be capable of regulating cell proliferation and differentiation of both normal and malignant cells (Reichel, H. et al. (1989) Ann. Rev. Med. 40: 71-78).

Given the activities of vitamin D_3 and its metabolites, much attention has focused on the development of synthetic analogues of these compounds. A large number of these analogues involve structural modifications in the A ring, B ring, C/D rings, and, primarily, the side chain (Bouillon, R. et al., Endocrine Reviews 16(2):201-204). Although a vast majority of the vitamin D_3 analogues developed to date involve structural modifications in the side chain, a few studies have reported the biological profile of A-ring diastereomers (Norman, A.W. et al. J. Biol. Chem. 268 (27): 20022-20030). Furthermore, biological esterification of steroids has been studied (Hochberg, R.B., (1998) Endocr Rev. 19(3): 331-348), and esters of vitamin D_3 are known (WO 97/11053).

Moreover, despite much effort in developing synthetic analogues, clinical applications of vitamin D and its structural analogues have been limited by the undesired side effects elicited by these compounds after administration to a subject for known indications/applications of vitamin D compounds.

The activated form of vitamin D, vitamin D3, and some of its analogues have been described as potent regulators of cell growth and differentiation. It has previously been found that vitamin D3 as well as an analogue (analogue V), inhibited BPH cell proliferation and counteracted the mitogenic activity of potent growth factors for BPH cells, such as keratinocyte growth factor (KGF).and insulin-like growth factor (IGF1). Moreover, the analogue induced bcl-2 protein expression, intracellular calcium mobilization, and apoptosis in both unstimulated and KGF-stimulated BPH cells

Furthermore, and as demonstrated in the Examples herein, calcitriol and other vitamin D analogues are effective in inhibiting the basal and stimulated growth of human bladder cells

Thus the invention provides Vitamin D compounds, and new methods of treatment using such compounds, for the prevention or treatment of bladder dysfunction.

Before further description of the present invention, and in order that the invention may be more readily understood, certain terms are first defined and collected here for convenience.

"By “bladder dysfunction" it is meant bladder conditions associated with overactivity of the detrusor muscle, for example, overactive bladder or clinical BPH. Whilst overactivity of the detrusor muscle is often associated with hypertrophy of the bladder, the treatment or prevention of overactivity via any cause is considered to be within the scope of the invention. Bladder dysfunction is usually characterised clinically by irritative (storage) symptoms. In current clinical practice, a diagnosis of overactive bladder is based upon the symptoms presented by the patient. Further urodynamic investigation may be used to confirm overactivity of the detrusor muscle.

The term "administration" or “administering” includes routes of introducing the vitamin D compound(s) to a subject to perform their intended function. Examples of routes of administration which can be used include injection (subcutaneous, intravenous, parenterally, intraperitoneally, oral, inhalation, rectal, transdermal or via bladder instillation. The pharmaceutical preparations are, of course, given by forms suitable for each administration route. For example, these preparations are administered in tablets or capsule form, by injection, inhalation, ointment, suppository, etc. administration by injection, infusion or inhalation; topical by lotion or ointment; and rectal by suppositories. Oral administration is preferred. The injection can be bolus or can be continuous infusion. Depending on the route of administration, the vitamin D compound can be coated with or disposed in a selected material to protect it from natural conditions which may detrimentally effect its ability to perform its intended function. The vitamin D compound can be administered alone, or in conjunction with either another agent as described above, for example with a smooth muscle relaxant (such as alpha blockers or anti-muscarinic drugs) or with a pharmaceutically-acceptable carrier, or both. The vitamin D compound can be administered prior to the administration of the other agent, simultaneously with the agent, or after the administration of the agent. Furthermore, the vitamin D compound can also be administered in a pro-form which is converted into its active metabolite, or more active metabolite *in vivo*.

The term “effective amount” includes an amount effective, at dosages and for periods of time necessary, to achieve the desired result, i.e. sufficient to treat bladder dysfunction. An effective amount of vitamin D compound may vary according to factors such as the disease state, age, gender and weight of the subject, and the ability of the vitamin D compound to elicit a desired response in the subject. Dosage regimens may be adjusted to provide the optimum therapeutic response. An effective amount is also one in which any

toxic or detrimental effects (*e.g.,* side effects) of the vitamin D compound are outweighed by the therapeutically beneficial effects.

A therapeutically effective amount of vitamin D compound (*i.e.*, an effective dosage) may range from about 0.001 to 30 μg/kg body weight, preferably about 0.01 to 25 μg/kg body weight, more preferably about 0.1 to 20 μg/kg body weight, and even more preferably about 1 to 10 μg/kg, 2 to 9 μg/kg, 3 to 8 μg/kg, 4 to 7 μg/kg, or 5 to 6 μg/kg body weight. The skilled artisan will appreciate that certain factors may influence the dosage required to effectively treat a subject, including but not limited to the severity of the disease or disorder, previous treatments, the general health and/or age of the subject, and other diseases present. In addition, the dose administered will also depend on the particular Vitamin D compound used, the effective amount of each compounds can be determined by titration methods known in the art. Moreover, treatment of a subject with a therapeutically effective amount of a vitamin D compound can include a single treatment or, preferably, can include a series of treatments. In one example, a subject is treated with a vitamin D compound in the range of between about 0.1 to 20 μg/kg body weight, one time per day for a duration of six months or longer, for example for life depending on management of the symptoms and the evolution of the condition. Also, as with other chronic treatments an "on-off" or intermittent treatment regime can be considered. It will also be appreciated that the effective dosage of a vitamin D compound used for treatment may increase or decrease over the course of a particular treatment.

The term "alkyl" refers to the radical of saturated aliphatic groups, including straight-chain alkyl groups, branched-chain alkyl groups, cycloalkyl (alicyclic) groups, alkyl substituted cycloalkyl groups, and cycloalkyl substituted alkyl groups. The term alkyl further includes alkyl groups, which can further include oxygen, nitrogen, sulfur or phosphorous atoms replacing one or more carbons of the hydrocarbon backbone, *e.g.,* oxygen, nitrogen,

sulfur or phosphorous atoms. In preferred embodiments, a straight chain or branched chain alkyl has 30 or fewer carbon atoms in its backbone (*e.g.,* C_1-C_{30} for straight chain, C_3-C_{30} for branched chain), preferably 26 or fewer, and more preferably 20 or fewer. Likewise, preferred cycloalkyls have from 3-10 carbon atoms in their ring structure, and more preferably have 3, 4, 5, 6 or 7 carbons in the ring structure.

Moreover, the term alkyl as used throughout the specification and claims is intended to include both "unsubstituted alkyls" and "substituted alkyls," the latter of which refers to alkyl moieties having substituents replacing a hydrogen on one or more carbons of the hydrocarbon backbone. Such substituents can include, for example, halogen, hydroxyl, alkylcarbonyloxy, arylcarbonyloxy, alkoxycarbonyloxy, aryloxycarbonyloxy, carboxylate, alkylcarbonyl, alkoxycarbonyl, aminocarbonyl, alkylthiocarbonyl, alkoxyl, phosphate, phosphonato, phosphinato, cyano, amino (including alkyl amino, dialkylamino, arylamino, diarylamino, and alkylarylamino), acylamino (including alkylcarbonylamino, arylcarbonylamino, carbamoyl and ureido), amidino, imino, sulfhydryl, alkylthio, arylthio, thiocarboxylate, sulfates, sulfonato, sulfamoyl, sulfonamido, nitro, trifluoromethyl, cyano, azido, heterocyclyl, alkylaryl, or an aromatic or heteroaromatic moiety. It will be understood by those skilled in the art that the moieties substituted on the hydrocarbon chain can themselves be substituted, if appropriate. Cycloalkyls can be further substituted, *e.g.,* with the substituents described above. An "alkylaryl" moiety is an alkyl substituted with an aryl (*e.g.,* phenylmethyl (benzyl)). The term “alkyl” also includes unsaturated aliphatic groups analogueous in length and possible substitution to the alkyls described above, but that contain at least one double or triple bond respectively.

Unless the number of carbons is otherwise specified, "lower alkyl" as used herein means an alkyl group, as defined above, but having from one to ten carbons, more preferably from one to six, and most preferably from one to

four carbon atoms in its backbone structure, which may be straight or branched-chain. Examples of lower alkyl groups include methyl, ethyl, n-propyl, i-propyl, tert-butyl, hexyl, heptyl, octyl and so forth. In preferred embodiment, the term "lower alkyl" includes a straight chain alkyl having 4 or fewer carbon atoms in its backbone, *e.g.,* C_1-C_4 alkyl.

The terms "alkoxyalkyl," "polyaminoalkyl" and "thioalkoxyalkyl" refer to alkyl groups, as described above, which further include oxygen, nitrogen or sulfur atoms replacing one or more carbons of the hydrocarbon backbone, *e.g.,* oxygen, nitrogen or sulfur atoms.

The term "aryl" as used herein, refers to the radical of aryl groups, including 5- and 6-membered single-ring aromatic groups that may include from zero to four heteroatoms, for example, benzene, pyrrole, furan, thiophene, imidazole, benzoxazole, benzothiazole, triazole, tetrazole, pyrazole, pyridine, pyrazine, pyridazine and pyrimidine, and the like. Aryl groups also include polycyclic fused aromatic groups such as naphthyl, quinolyl, indolyl, and the like. Those aryl groups having heteroatoms in the ring structure may also be referred to as "aryl heterocycles," "heteroaryls" or "heteroaromatics." The aromatic ring can be substituted at one or more ring positions with such substituents as described above, as for example, halogen, hydroxyl, alkoxy, alkylcarbonyloxy, arylcarbonyloxy, alkoxycarbonyloxy, aryloxycarbonyloxy, carboxylate, alkylcarbonyl, alkoxycarbonyl, aminocarbonyl, alkylthiocarbonyl, phosphate, phosphonato, phosphinato, cyano, amino (including alkyl amino, dialkylamino, arylamino, diarylamino, and alkylarylamino), acylamino (including alkylcarbonylamino, arylcarbonylamino, carbamoyl and ureido), amidino, imino, sulfhydryl, alkylthio, arylthio, thiocarboxylate, sulfates, sulfonato, sulfamoyl, sulfonamido, nitro, trifluoromethyl, cyano, azido, heterocyclyl, alkylaryl, or an aromatic or heteroaromatic moiety. Aryl groups can also be fused or bridged with alicyclic

or heterocyclic rings which are not aromatic so as to form a polycycle (*e.g.,* tetralin).

The terms "alkenyl" and "alkynyl" refer to unsaturated aliphatic groups analogueous in length and possible substitution to the alkyls described above, but that contain at least one double or triple bond, respectively. For example, the invention contemplates cyano and propargyl groups.

The term "chiral" refers to molecules which have the property of non-superimposability of the mirror image partner, while the term "achiral" refers to molecules which are superimposable on their mirror image partner.

The term "diastereomers" refers to stereoisomers with two or more centers of dissymmetry and whose molecules are not mirror images of one another.

The term "enantiomers" refers to two stereoisomers of a compound which are non-superimposable mirror images of one another. An equimolar mixture of two enantiomers is called a "racemic mixture" or a "racemate."

As used herein, the term "halogen" designates -F, -Cl, -Br or -I; the term "sulfhydryl" or "thiol" means -SH; the term "hydroxyl" means -OH.

The term "haloalkyl" is intended to include alkyl groups as defined above that are mono-, di- or polysubstituted by halogen, *e.g.*, fluoromethyl and trifluoromethyl.

The term "heteroatom" as used herein means an atom of any element other than carbon or hydrogen. Preferred heteroatoms are nitrogen, oxygen, sulfur and phosphorus.

The terms "polycyclyl" or "polycyclic radical" refer to the radical of two or more cyclic rings (*e.g.,* cycloalkyls, cycloalkenyls, cycloalkynyls, aryls and/or heterocyclyls) in which two or more carbons are common to two adjoining rings, *e.g.,* the rings are "fused rings". Rings that are joined through non-adjacent atoms are termed "bridged" rings. Each of the rings of the polycycle can be substituted with such substituents as described above, as for example, halogen, hydroxyl, alkylcarbonyloxy, arylcarbonyloxy, alkoxycarbonyloxy,

aryloxycarbonyloxy, carboxylate, alkylcarbonyl, alkoxycarbonyl, aminocarbonyl, alkylthiocarbonyl, alkoxyl, phosphate, phosphonato, phosphinato, cyano, amino (including alkyl amino, dialkylamino, arylamino, diarylamino, and alkylarylamino), acylamino (including alkylcarbonylamino, arylcarbonylamino, carbamoyl and ureido), amidino, imino, sulfhydryl, alkylthio, arylthio, thiocarboxylate, sulfates, sulfonato, sulfamoyl, sulfonamido, nitro, trifluoromethyl, cyano, azido, heterocyclyl, alkyl, alkylaryl, or an aromatic or heteroaromatic moiety.

The term "isomers" or "stereoisomers" refers to compounds which have identical chemical constitution, but differ with regard to the arrangement of the atoms or groups in space.

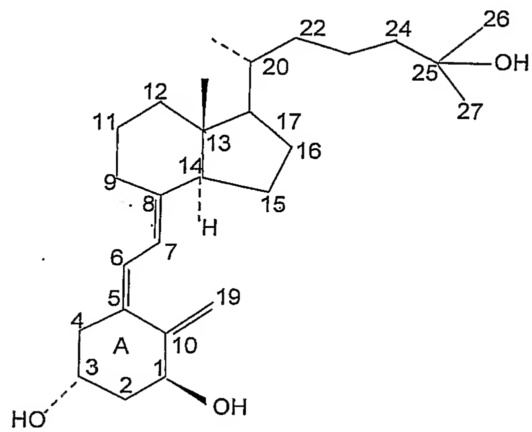
The terms "isolated" or "substantially purified" are used interchangeably herein and refer to vitamin D_3 compounds in a non-naturally occurring state. The compounds can be substantially free of cellular material or culture medium when naturally produced, or chemical precursors or other chemicals when chemically synthesized. In certain preferred embodiments, the terms "isolated" or "substantially purified" also refer to preparations of a chiral compound which substantially lack one of the enantiomers; *i.e.*, enantiomerically enriched or non-racemic preparations of a molecule. Similarly, the terms "isolated epimers" or "isolated diastereomers" refer to preparations of chiral compounds which are substantially free of other stereochemical forms. For instance, isolated or substantially purified vitamin D_3 compounds include synthetic or natural preparations of a vitamin D_3 enriched for the stereoisomers having a substituent attached to the chiral carbon at position 3 of the A-ring in an α (alpha) -configuration, and thus substantially lacking other isomers having a β (beta) -configuration. Unless otherwise specified, such terms refer to vitamin D_3 compositions in which the ratio of α to β forms is greater than 1:1 by weight. For instance, an isolated preparation of an a epimer means a preparation having greater than 50% by

weight of the α-epimer relative to the β stereoisomer, more preferably at least 75% by weight, and even more preferably at least 85% by weight. Of course the enrichment can be much greater than 85%, providing "substantially epimer-enriched" preparations, *i.e.*, preparations of a compound which have greater than 90% of the α-epimer relative to the β stereoisomer, and even more preferably greater than 95%. The term "substantially free of the β stereoisomer" will be understood to have similar purity ranges.

As used herein, the term "vitamin D compound" includes any compound that is capable of treating or preventing bladder dysfunction. Generally, compounds which are ligands for the Vitamin D receptor (VDR ligands) and which are capable of treating or preventing bladder dysfunction are considered to be within the scope of the invention. Thus, vitamin D compounds are intended to include secosteroids. Examples of specific vitamin D compounds suitable for use in the methods of the present invention are further described herein. A vitamin D compound includes vitamin D_2 compounds, vitamin D_3 compounds, isomers thereof, or derivatives/analogues thereof. In certain embodiments, the vitamin D compound may be a steroid, such as a secosteroid, *e.g.,* calciol, calcidiol or calcitriol. Non-limiting examples of vitamin D compounds in accordance with the invention include those described in U.S. Patent Nos. 6,017,908, 6,100,294, 6,030,962, 5,428029 and 6,121,312, published international applications WO 98/51633, WO 01/40177A3.

The term "secosteroid" is art-recognized and includes compounds in which one of the cyclopentanoperhydro- phenanthrene rings of the steroid ring structure is broken. For example, $1\alpha,25(OH)_2D_3$ and analogues thereof are hormonally active secosteroids. In the case of vitamin D_3, the 9-10 carbon-carbon bond of the B-ring is broken, generating a seco-B-steroid. The official IUPAC name for vitamin D_3 is 9,10-secocholesta-5,7,10(19)-trien-3B-ol. For

convenience, a 6-s-*trans* conformer of $1\alpha,25(OH)_2D_3$ is illustrated herein having all carbon atoms numbered using standard steroid notation.

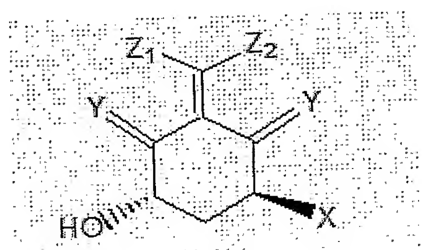


In the formulas presented herein, the various substituents on ring A are illustrated as joined to the steroid nucleus by one of these notations: a dotted line (----) indicating a substituent which is in the β-orientation (*i.e.* , above the plane of the ring), a wedged solid line (◄) indicating a substituent which is in the α-orientation (*i.e.* , below the plane of the molecule), or a wavy line (∿∿∿) indicating that a substituent may be either above or below the plane of the ring. In regard to ring A, it should be understood that the stereochemical convention in the vitamin D field is opposite from the general chemical field, wherein a dotted line indicates a substituent on Ring A which is in an α-orientation (*i.e.* , below the plane of the molecule), and a wedged solid line indicates a substituent on ring A which is in the β-orientation (*i.e.* , above the plane of the ring). As shown, the A ring of the hormone $1\alpha,25(OH)_2D_3$ contains two asymmetric centers at carbons 1 and 3, each one containing a hydroxyl group in well-characterized configurations, namely the 1α- and 3β-hydroxyl groups. In other words, carbons 1 and 3 of the A ring are said to be "chiral carbons" or "carbon centers."

With respect to the nomenclature of a chiral center, terms "d" and "l" configuration are as defined by the IUPAC Recommendations. As to the use of the terms, diastereomer, racemate, epimer and enantiomer will be used in their normal context to describe the stereochemistry of preparations.

Thus, in one aspect, the invention provides the use of a Vitamin D compound in the prevention or treatment of bladder dysfunction. Also provided is a method of treating a patient with bladder dysfunction by administering a effective amount of a Vitamin D compound. Further provided is the use of a Vitamin D compound in the manufacture of a medicament for the prevention or treatment of bladder dysfunction.

In one embodiment of the invention, the vitamin D compound comprises formula I:



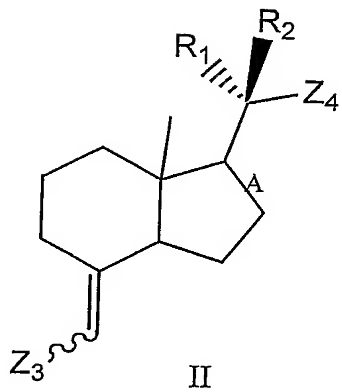
I

wherein

X is hydroxyl or fluoro;

Y is H_2 or CH_2;

Z_1 and Z_2 are H or a substituent represented by formula II, provided Z_1 and Z_2 are different:

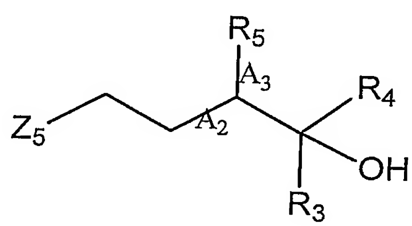


II

wherein Z_3 represents the above-described formula I;

A is a single bond or a double bond;

R_1, R_2, and Z_4, are each, independently, hydrogen, alkyl, or a saturated or unsaturated carbon chain represented by formula III, provided that at least one of R_1, R_2, and Z_4 is the saturated or unsaturated carbon chain represented by formula III and provided that all of R_1, R_2, and Z_4 are not saturated or unsaturated carbon chain represented by formula III:



III

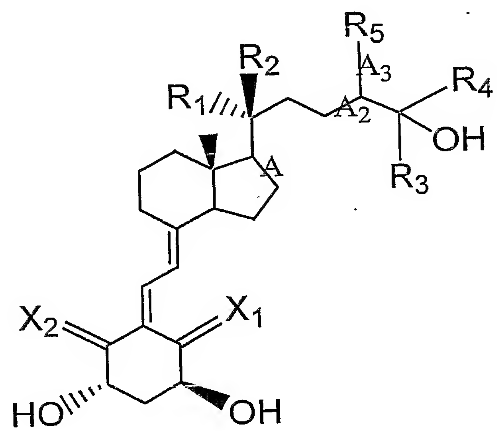
wherein Z_5 represents the above-described formula II;

A_2 is a single bond, a double bond, or a triple bond; and

A_3 is a single bond or a double bond; and

R_3, and R_4, are each, independently, hydrogen, alkyl, haloalkyl, hydroxyalkyl; and R_5 is H_2 or oxygen.

In another embodiment of the invention, the vitamin D compound is a compound of formula:



wherein:

X_1 and X_2 are H_2 or CH_2, wherein X_1 and X_2 are not CH_2 at the same time;

A is a single or double bond;

A_2 is a single, double or triple bond;

A_3 is a single or double bond;

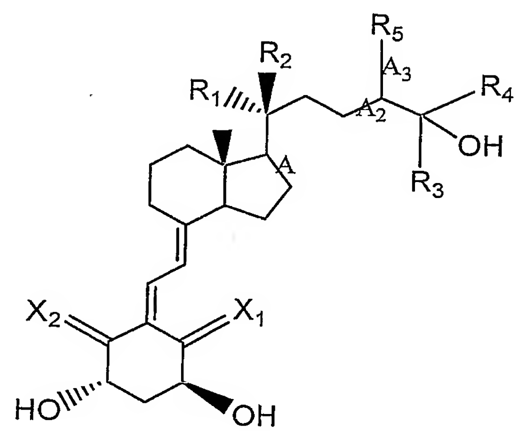
R_1 and R_2 are hydrogen, C_1-C_4 alkyl or 4-hydroxy-4-methylpentyl, wherein R_1 and R_2 are not both hydrogen;

R_5 is H_2 or oxygen,;

R_3 is C_1-C_4 alkyl, hydroxyalkyl or haloalkyl, *eg.*, fluoroalkyl,*e.g.*, fluoromethyl and trifluoromethyl; and

R_4 is C_1-C_4 alkyl, hydroxyalkyl or haloalkyl, *eg.*, fluoroalkyl,*e.g.*, fluoromethyl and trifluoromethyl.

In yet another embodiment of the invention, the vitamin D compound is a compound of the formula:



wherein:

X_1 and X_2 are H_2 or CH_2, wherein X_1 and X_2 are not CH_2 at the same time;

A is a single or double bond;

A_2 is a single, double or triple bond;

A_3 is a single or double bond;

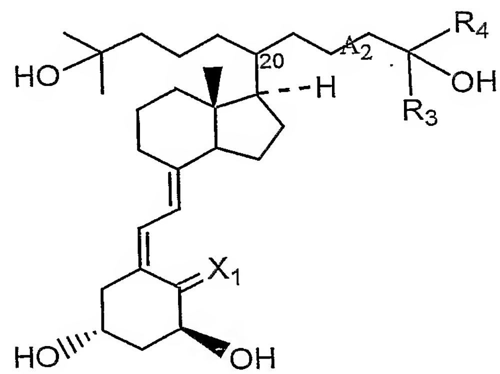
R_1 and R_2 are hydrogen, C_1-C_4 alkyl, wherein R_1 and R_2 are not both hydrogen;

R_5 is H_2 or oxygen,;

R_3 is C_1-C_4 alkyl, hydroxyalkyl or haloalkyl, *e.g.*, fluoroalkyl, *e.g.*, fluoromethyl and trifluoromethyl; and

R_4 is C_1-C_4 alkyl, hydroxyalkyl haloalkyl, *e.g.*, or fluoroalkyl, *e.g.*, fluoromethyl and trifluoromethyl.

In yet another embodiment, the vitamin D compound is a compound of the formula:



wherein:

X_1 is H_2 or CH_2;

A_2 is a single, a double or a triple bond;

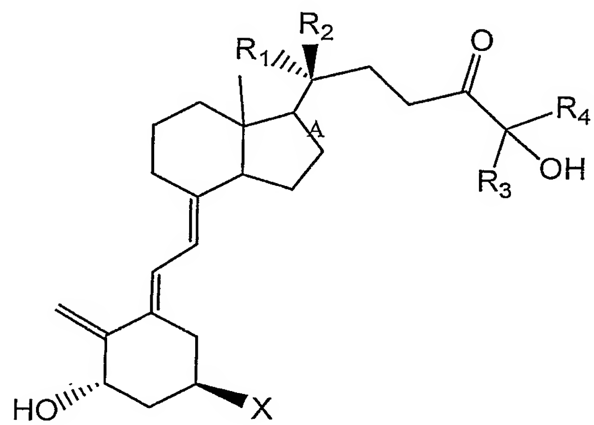
R_3 is C_1-C_4 alkyl, hydroxyalkyl, or haloalkyl, *e.g.*, fluoroalkyl, *e.g.*, fluoromethyl and trifluoromethyl;

R_4 is C_1-C_4 alkyl, hydroxyalkyl or haloalkyl, *e.g.*, fluoroalkyl, *e.g.*, fluoromethyl and trifluoromethyl;

and

the configuration at C_{20} is R or S.

In another embodiment, the vitamin D compound is a compound of the formula:



wherein:

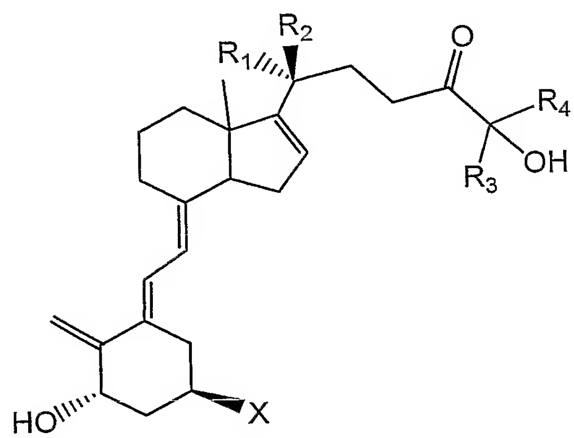
A is a single or double bond;

R_1 and R_2 are each, independently, hydrogen, alkyl;

R_3, and R_4, are each, independently, alkyl, and

X is hydroxyl or fluoro.

In a further embodiment, the vitamin D compound is a compound having formula:



wherein:

R_1 and R_2, are each, independently, hydrogen, or alkyl, *e.g.*, methyl;

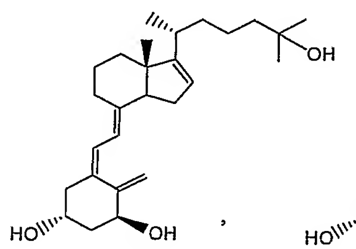
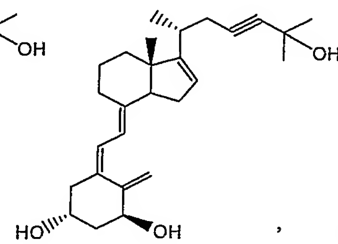
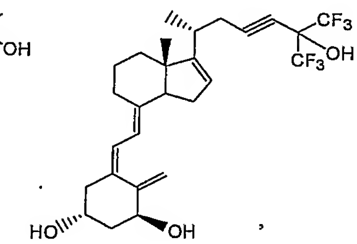
R_3 is alkyl, *e.g.*, methyl,

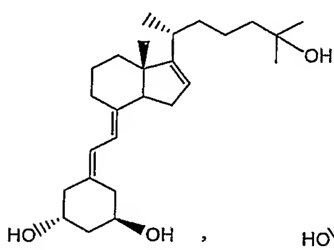
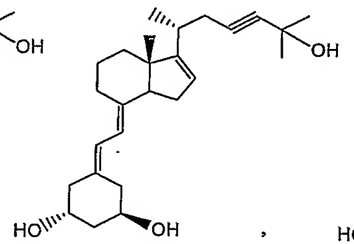
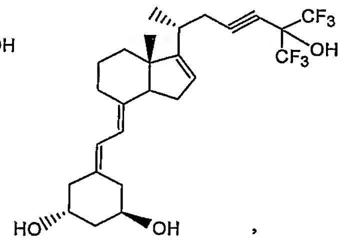
R_4 is alkyl, *e.g.*, methyl;

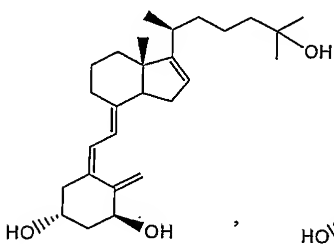
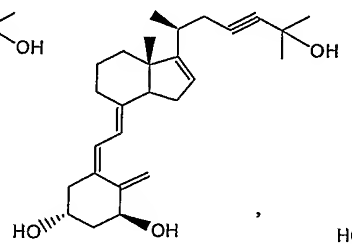
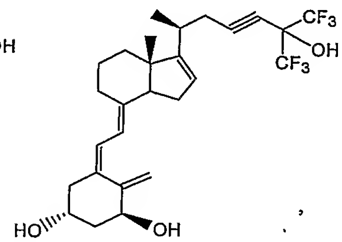
and

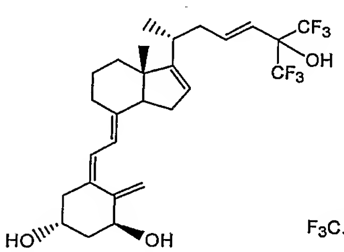
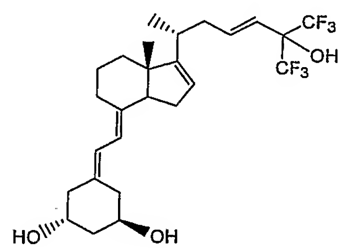
X is hydroxyl or fluoro.

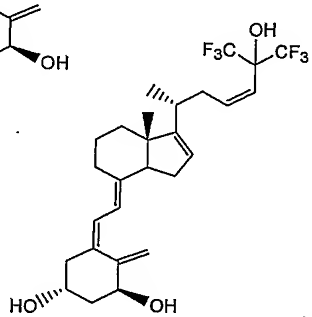
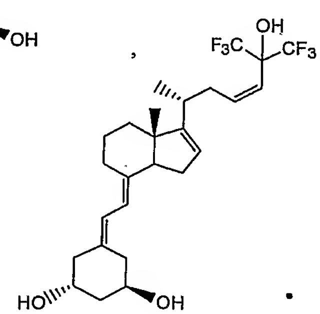
In specific embodiments of the invention, the vitamin D compound is selected from the group consisting of

 ,  ,  ,

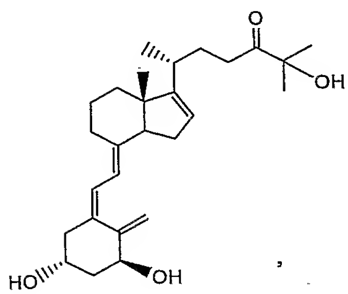
 ,  ,  ,

 ,  ,  ,

 ,  ,

 , and  .

In other specific embodiments of the invention, the vitamin D compound is selected from the group consisting of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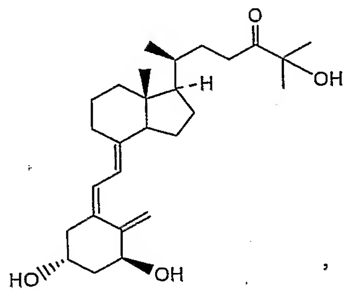
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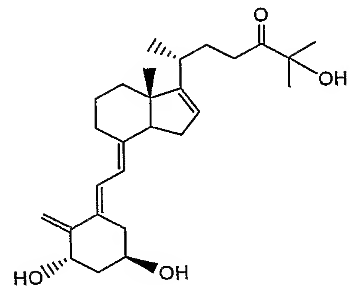

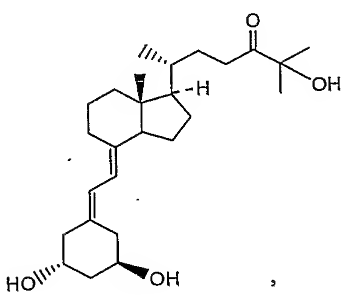
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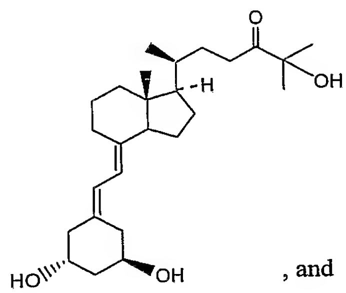
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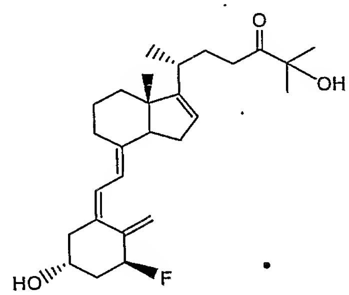
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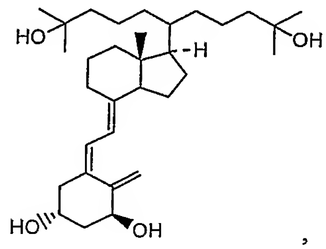
In further specific embodiments of the invention, the vitamin D compound is selected from the group consisting of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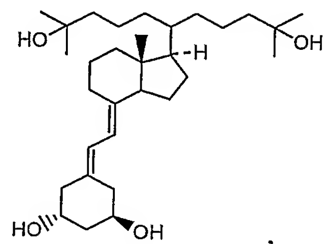
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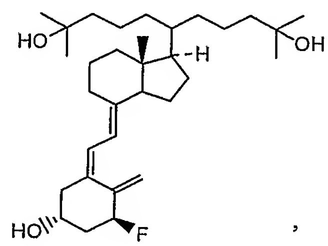
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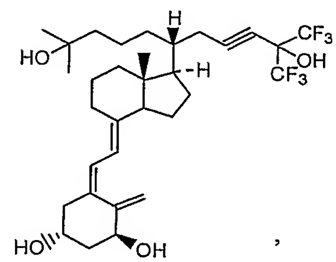
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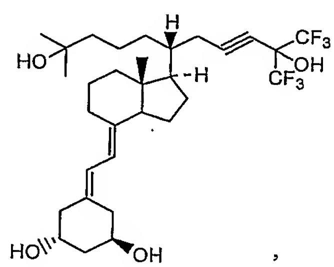
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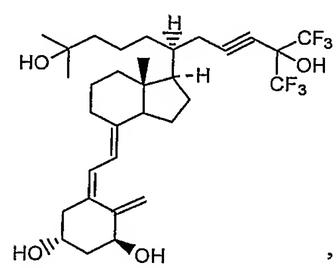
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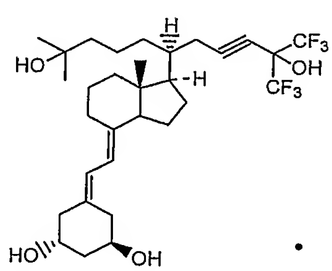
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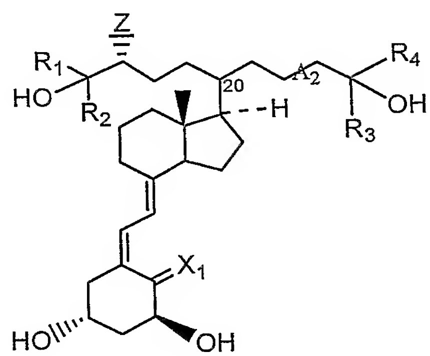
In still further specific embodiments of the invention, the vitamin D compound is a compound of the formula



wherein:

X_1 is H_2 or CH_2;

A_2 is a single, a double or a triple bond;

R_1, R_2, R_3 and R_4 are each independently C_1-C_4 alkyl, hydroxyalkyl, or haloalkyl, *e.g.*, fluoroalkyl, *e.g.*, fluoromethyl and trifluoromethyl;

Z is –OH;

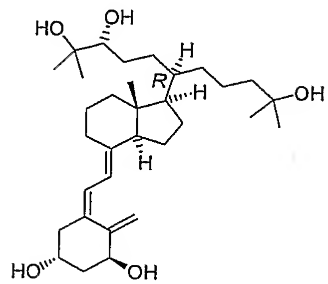
and

the configuration at C_{20} is R or S,

and pharmaceutically acceptable esters, salts, and prodrugs thereof. Compounds of this formula may be referred to as "geminal vitamin D_3" compounds due to the presence of two alkyl chains at C20.

In a further embodiment, X_1 is CH_2. In another embodiment, A_2 is a single bond. In another, R_1, R_2, R_3, and R_4 are each independently methyl or ethyl. In a further embodiment, Z is –OH. In another, X_1 is CH_2; A_2 is a single bond; R_1, R_2, R_3, and R_4 are each independently methyl or ethyl; and Z is –OH. In an even further embodiment, R_1, R_2, R_3, and R_4 are each methyl.

In a further embodiment of the invention, the vitamin D compound is a compound of the formula:



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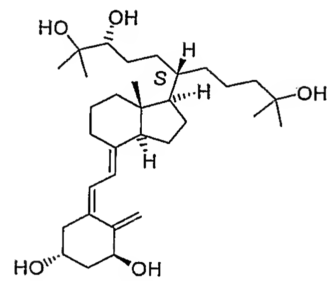
or



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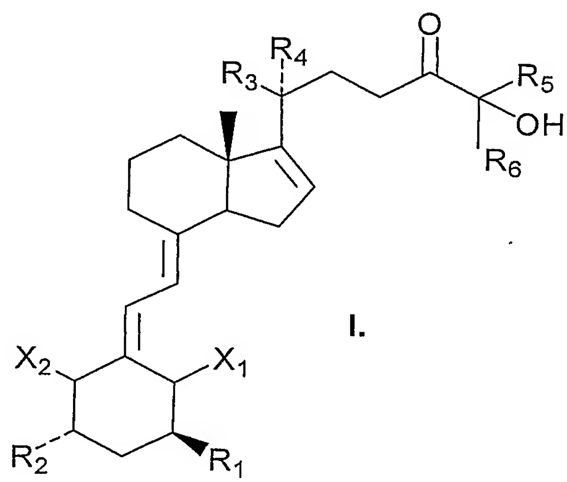
In further embodiments of the invention, the vitamin D compound is a compound of the formula:



I.

wherein:

X_1 and X_1 are each independently H_2 or $=CH_2$, provided X_1 and X_1 are not both $=CH_2$;

R_1 and R_2 are each independently, hydroxyl, $OC(O)C_1$-C_4 alkyl, OC(O)hydroxyalkyl, OC(O)fluroralkyl;

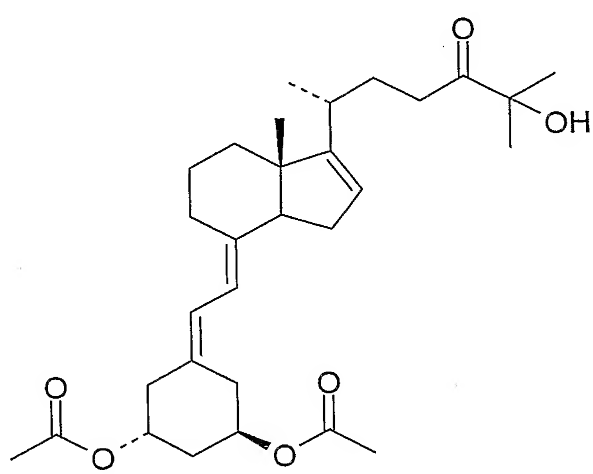
R_3 and R_4 are each independently hydrogen, C_1-C_4 alkyl, or R_3 and R_4 taken together with C_{20} form C_3-C_6 cylcoalkyl; and

R_5 and R_6 are each independently C_1-C_4 alkyl and pharmaceutically acceptable esters, salts, and prodrugs thereof.

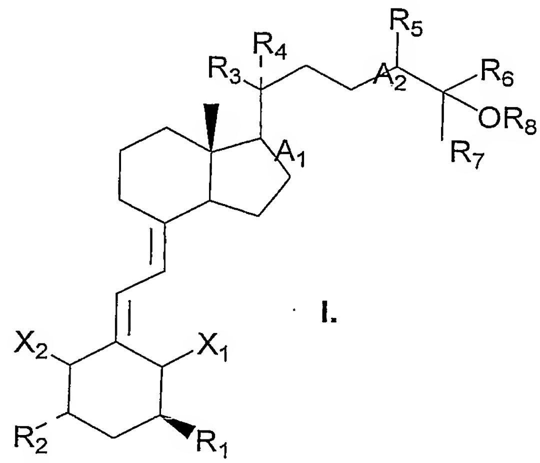
In one embodiment, X_1 and X_1 are each H_2. In another embodiment, R_3 is hydrogen and R_4 is C_1-C_4 alkyl. In a preferred embodiment R_4 is methyl. In another embodiment, R_5 and R_6 are each independently methyl, ethyl fluoromethyl or trifluoromethyl. In a preferred embodiment, R_5 and R_6 are each methyl.

In yet another embodiment, R_1 and R_1 are each independently hydroxyl or $OC(O)C_1$-C_4 alkyl. In a preferred embodiment, R_1 and R_1 are each $OC(O)C_1$-C_4 alkyl. In another preferred embodiment, R_1 and R_1 are each acetyloxy.

An example of such a compound is 1,3-O-diacetyl-1,25-diydroxy-16-ene-24-keto-19-nor-cholecalciferol, having the following structure:



In another embodiment of the invention, the vitamin D compound is a compound of the formula:



I.

wherein:

A_1 is single or double bond;

A_2 is a single, double or triple bond;

X_1 and X_2 are each independently H or $=CH_2$, provided X_1 and X_2 are not both $=CH_2$;

R_1 and R_2 are each independently $OC(O)C_1$-C_4 alkyl, OC(O)hydroxyalkyl, OROC(O)haloalkyl, OAc;

R_3, R_4 and R_5 are each independently hydrogen, C_1-C_4 alkyl, hydroxyalkyl, or haloalkyl, or R_3 and R_4 taken together with C_{20} form C_3-C_6 cylcoalkyl; and

R_6 and R_7 are each independently haloalkyl; and

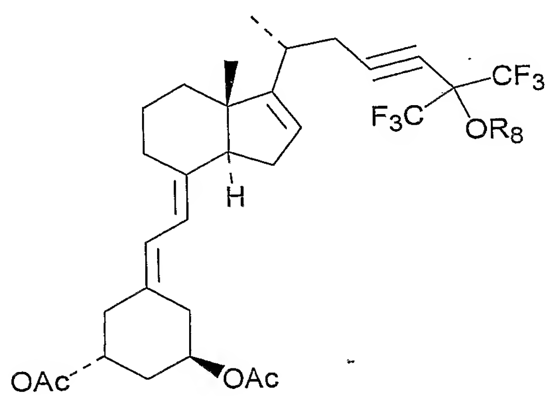
R_8 is H or Ac; and

pharmaceutically acceptable esters, salts, and prodrugs thereof.

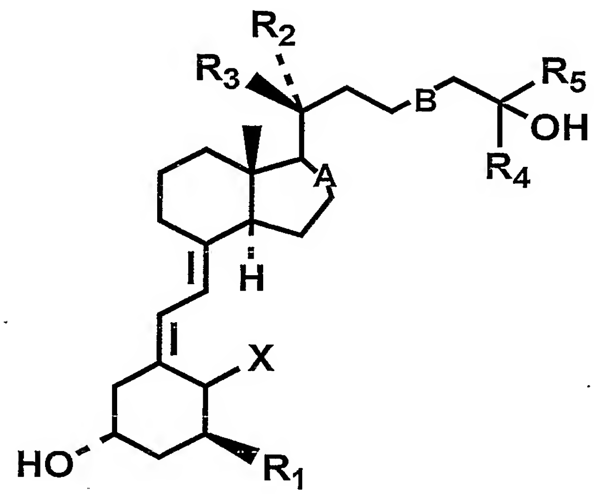
In one embodiment, X_1 and X_2 are each H. In another embodiment, R_3 is hydrogen and R_4 is C_1-C_4 alkyl. In a preferred embodiment R_4 is methyl.

In another embodiment, R_6 and R_7 are each independently methyl, ethyl or fluoroalkyl. In a preferred embodiment, R_6 and R_8 are each trifluoroalkyl, *e.g.*, trifluoromethyl.

In another preferred embodiment, R_1 and R_2 are each OAc; A_1 is a double bond; A_2 is a triple bond; and R_8 is either H or OAc:



In a more preferred embodiment, vitamin D compounds for use in the invention are compounds of the formula



wherein

X is H_2 or CH_2

R_1 is hydrogen, hydroxy or fluorine

R_2 is hydrogen or methyl

R_3 is hydrogen or methyl. When R_2 or R_3 is methyl, R_3 or R_2 must be hydrogen.

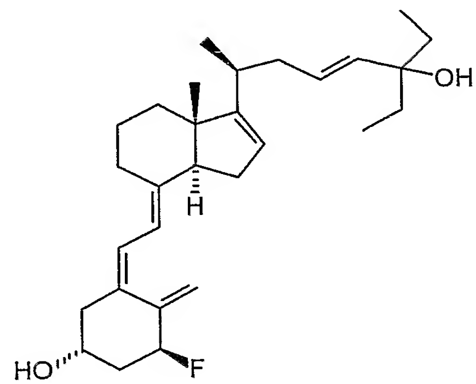
R_4 is methyl, ethyl or trifluoromethyl

R_5 is methyl, ethyl or trifluoromethyl

A is a single or double bond

B is a single, E-double, Z-double or triple bond

In particularly preferred compounds, each of R_4 and R_5 is methyl or ethyl, for example 1-alpha-fluoro-25-hydroxy-16,23E-diene-26,27-bishomo-20-epi-cholecalciferol (compound A in the following examples), having the formula:



Such compounds are described in US 5,939,408.

It will be noted that the structures of some of the compounds of the invention include asymmetric carbon atoms. Accordingly, it is to be understood that the isomers arising from such asymmetry (*e.g.*, all enantiomers and diastereomers) are included within the scope of this invention, unless indicated otherwise. Such isomers can be obtained in substantially pure form by classical separation techniques and/or by stereochemically controlled synthesis.

Naturally occurring or synthetic isomers can be separated in several ways known in the art. Methods for separating a racemic mixture of two enantiomers include chromatography using a chiral stationary phase (see, *e.g.*, , "Chiral Liquid Chromatography," W.J. Lough, Ed. Chapman and Hall, New York (1989)). Enantiomers can also be separated by classical resolution techniques. For example, formation of diastereomeric salts and fractional crystallization can be used to separate enantiomers. For the separation of

enantiomers of carboxylic acids, the diastereomeric salts can be formed by addition of enantiomerically pure chiral bases such as brucine, quinine, ephedrine, strychnine, and the like. Alternatively, diastereomeric esters can be formed with enantiomerically pure chiral alcohols such as menthol, followed by separation of the diastereomeric esters and hydrolysis to yield the free, enantiomerically enriched carboxylic acid. For separation of the optical isomers of amino compounds, addition of chiral carboxylic or sulfonic acids, such as camphorsulfonic acid, tartaric acid, mandelic acid, or lactic acid can result in formation of the diastereomeric salts.

The invention also provides a pharmaceutical composition, comprising an effective amount of a vitamin D compound as described herein and a pharmaceutically acceptable carrier. In a further embodiment, the effective amount is effective to treat bladder dysfunction, as described previously.

In an embodiment, the vitamin D compound is administered to the subject using a pharmaceutically-acceptable formulation, e.g., a pharmaceutically-acceptable formulation that provides sustained delivery of the vitamin D compound to a subject for at least 12 hours, 24 hours, 36 hours, 48 hours, one week, two weeks, three weeks, or four weeks after the pharmaceutically-acceptable formulation is administered to the subject.

In certain embodiments, these pharmaceutical compositions are suitable for topical or oral administration to a subject. In other embodiments, as described in detail below, the pharmaceutical compositions of the present invention may be specially formulated for administration in solid or liquid form, including those adapted for the following: (1) oral administration, for example, drenches (aqueous or non-aqueous solutions or suspensions), tablets, boluses, powders, granules, pastes; (2) parenteral administration, for example, by subcutaneous, intramuscular or intravenous injection as, for example, a sterile solution or suspension, (3) topical application, for example, as a cream, ointment or spray applied to the skin; (4) intravaginally or intrarectally, for example, as a pessary, cream or foam; or (5) aerosol, for example, as an

aqueous aerosol, liposomal preparation or solid particles containing the compound.

The phrase "pharmaceutically acceptable" refers to those vitamin D compounds of the present invention, compositions containing such compounds, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

The phrase "pharmaceutically-acceptable carrier" includes pharmaceutically-acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, solvent or encapsulating material, involved in carrying or transporting the subject chemical from one organ, or portion of the body, to another organ, or portion of the body. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the patient. Some examples of materials which can serve as pharmaceutically-acceptable carriers include: (1) sugars, such as lactose, glucose and sucrose; (2) starches, such as corn starch and potato starch; (3) cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; (4) powdered tragacanth; (5) malt; (6) gelatin; (7) talc; (8) excipients, such as cocoa butter and suppository waxes; (9) oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; (10) glycols, such as propylene glycol; (11) polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; (12) esters, such as ethyl oleate and ethyl laurate; (13) agar; (14) buffering agents, such as magnesium hydroxide and aluminum hydroxide; (15) alginic acid; (16) pyrogen-free water; (17) isotonic saline; (18) Ringer's solution; (19) ethyl alcohol; (20) phosphate buffer solutions; and (21) other non-toxic compatible substances employed in pharmaceutical formulations.

Wetting agents, emulsifiers and lubricants, such as sodium lauryl sulfate and magnesium stearate, as well as coloring agents, release agents,

coating agents, sweetening, flavoring and perfuming agents, preservatives and antioxidants can also be present in the compositions.

Examples of pharmaceutically-acceptable antioxidants include: (1) water soluble antioxidants, such as ascorbic acid, cysteine hydrochloride, sodium bisulfate, sodium metabisulfite, sodium sulfite and the like; (2) oil-soluble antioxidants, such as ascorbyl palmitate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), lecithin, propyl gallate, alpha-tocopherol, and the like; and (3) metal chelating agents, such as citric acid, ethylenediamine tetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric acid, and the like.

Compositions containing a vitamin D compound(s) include those suitable for oral, nasal, topical (including buccal and sublingual), rectal, vaginal, aerosol and/or parenteral administration. The compositions may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will vary depending upon the host being treated, the particular mode of administration. The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will generally be that amount of the compound which produces a therapeutic effect. Generally, out of one hundred per cent, this amount will range from about 1 per cent to about ninety-nine percent of active ingredient, preferably from about 5 per cent to about 70 per cent, most preferably from about 10 per cent to about 30 per cent.

Methods of preparing these compositions include the step of bringing into association a vitamin D compound(s) with the carrier and, optionally, one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing into association a vitamin D compound with liquid carriers, or finely divided solid carriers, or both, and then, if necessary, shaping the product.

Compositions of the invention suitable for oral administration may be in the form of capsules, cachets, pills, tablets, lozenges (using a flavored basis, usually sucrose and acacia or tragacanth), powders, granules, or as a solution or a suspension in an aqueous or non-aqueous liquid, or as an oil-in-water or water-in-oil liquid emulsion, or as an elixir or syrup, or as pastilles (using an inert base, such as gelatin and glycerin, or sucrose and acacia) and/or as mouth washes and the like, each containing a predetermined amount of a vitamin D compound(s) as an active ingredient. A compound may also be administered as a bolus, electuary or paste.

In solid dosage forms of the invention for oral administration (capsules, tablets, pills, dragees, powders, granules and the like), the active ingredient is mixed with one or more pharmaceutically-acceptable carriers, such as sodium citrate or dicalcium phosphate, and/or any of the following: (1) fillers or extenders, such as starches, lactose, sucrose, glucose, mannitol, and/or silicic acid; (2) binders, such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinyl pyrrolidone, sucrose and/or acacia; (3) humectants, such as glycerol; (4) disintegrating agents, such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate; (5) solution retarding agents, such as paraffin; (6) absorption accelerators, such as quaternary ammonium compounds; (7) wetting agents, such as, for example, acetyl alcohol and glycerol monostearate; (8) absorbents, such as kaolin and bentonite clay; (9) lubricants, such a talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof; and (10) coloring agents. In the case of capsules, tablets and pills, the pharmaceutical compositions may also comprise buffering agents. Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugars, as well as high molecular weight polyethylene glycols and the like.

A tablet may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared using

binder (for example, gelatin or hydroxypropylmethyl cellulose), lubricant, inert diluent, preservative, disintegrant (for example, sodium starch glycolate or cross-linked sodium carboxymethyl cellulose), surface-active or dispersing agent. Molded tablets may be made by molding in a suitable machine a mixture of the powdered active ingredient moistened with an inert liquid diluent.

The tablets, and other solid dosage forms of the pharmaceutical compositions of the present invention, such as dragees, capsules, pills and granules, may optionally be scored or prepared with coatings and shells, such as enteric coatings and other coatings well known in the pharmaceutical-formulating art. They may also be formulated so as to provide slow or controlled release of the active ingredient therein using, for example, hydroxypropylmethyl cellulose in varying proportions to provide the desired release profile, other polymer matrices, liposomes and/or microspheres. They may be sterilized by, for example, filtration through a bacteria-retaining filter, or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved in sterile water, or some other sterile injectable medium immediately before use. These compositions may also optionally contain opacifying agents and may be of a composition that they release the active ingredient(s) only, or preferentially, in a certain portion of the gastrointestinal tract, optionally, in a delayed manner. Examples of embedding compositions which can be used include polymeric substances and waxes. The active ingredient can also be in micro-encapsulated form, if appropriate, with one or more of the above-described excipients.

Liquid dosage forms for oral administration of the vitamin D compound(s) include pharmaceutically-acceptable emulsions, microemulsions, solutions, suspensions, syrups and elixirs. In addition to the active ingredient, the liquid dosage forms may contain inert diluents commonly used in the art, such as, for example, water or other solvents, solubilizing agents and emulsifiers, such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl

acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor and sesame oils), glycerol, tetrahydrofuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof.

In addition to inert diluents, the oral compositions can include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, coloring, perfuming and preservative agents.

Suspensions, in addition to the active vitamin D compound(s) may contain suspending agents as, for example, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar and tragacanth, and mixtures thereof.

Pharmaceutical compositions of the invention for rectal or vaginal administration may be presented as a suppository, which may be prepared by mixing one or more vitamin D compound(s) with one or more suitable nonirritating excipients or carriers comprising, for example, cocoa butter, polyethylene glycol, a suppository wax or a salicylate, and which is solid at room temperature, but liquid at body temperature and, therefore, will melt in the rectum or vaginal cavity and release the active agent.

Compositions of the present invention which are suitable for vaginal administration also include pessaries, tampons, creams, gels, pastes, foams or spray formulations containing such carriers as are known in the art to be appropriate.

Dosage forms for the topical or transdermal administration of a vitamin D compound(s) include powders, sprays, ointments, pastes, creams, lotions, gels, solutions, patches and inhalants. The active vitamin D compound(s) may be mixed under sterile conditions with a pharmaceutically-acceptable carrier, and with any preservatives, buffers, or propellants which may be required.

The ointments, pastes, creams and gels may contain, in addition to vitamin D compound(s) of the present invention, excipients, such as animal

and vegetable fats, oils, waxes, paraffins, starch, tragacanth, cellulose derivatives, polyethylene glycols, silicones, bentonites, silicic acid, talc and zinc oxide, or mixtures thereof.

Powders and sprays can contain, in addition to a vitamin D compound(s), excipients such as lactose, talc, silicic acid, aluminum hydroxide, calcium silicates and polyamide powder, or mixtures of these substances. Sprays can additionally contain customary propellants, such as chlorofluorohydrocarbons and volatile unsubstituted hydrocarbons, such as butane and propane.

The vitamin D compound(s) can be alternatively administered by aerosol. This is accomplished by preparing an aqueous aerosol, liposomal preparation or solid particles containing the compound. A nonaqueous (*e.g.,* fluorocarbon propellant) suspension could be used. Sonic nebulizers are preferred because they minimize exposing the agent to shear, which can result in degradation of the compound.

Ordinarily, an aqueous aerosol is made by formulating an aqueous solution or suspension of the agent together with conventional pharmaceutically-acceptable carriers and stabilizers. The carriers and stabilizers vary with the requirements of the particular compound, but typically include nonionic surfactants (Tweens, Pluronics, or polyethylene glycol), innocuous proteins like serum albumin, sorbitan esters, oleic acid, lecithin, amino acids such as glycine, buffers, salts, sugars or sugar alcohols. Aerosols generally are prepared from isotonic solutions.

Transdermal patches have the added advantage of providing controlled delivery of a vitamin D compound(s) to the body. Such dosage forms can be made by dissolving or dispersing the agent in the proper medium. Absorption enhancers can also be used to increase the flux of the active ingredient across the skin. The rate of such flux can be controlled by either providing a rate controlling membrane or dispersing the active ingredient in a polymer matrix or gel.

Pharmaceutical compositions of the invention suitable for parenteral administration comprise one or more vitamin D compound(s) in combination with one or more pharmaceutically-acceptable sterile isotonic aqueous or nonaqueous solutions, dispersions, suspensions or emulsions, or sterile powders which may be reconstituted into sterile injectable solutions or dispersions just prior to use, which may contain antioxidants, buffers, bacteriostats, solutes which render the formulation isotonic with the blood of the intended recipient or suspending or thickening agents.

Examples of suitable aqueous and nonaqueous carriers which may be employed in the pharmaceutical compositions of the invention include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

These compositions may also contain adjuvants such as preservatives, wetting agents, emulsifying agents and dispersing agents. Prevention of the action of microorganisms may be ensured by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol sorbic acid, and the like. It may also be desirable to include isotonic agents, such as sugars, sodium chloride, and the like into the compositions. In addition, prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents which delay absorption such as aluminum monostearate and gelatin.

In some cases, in order to prolong the effect of a drug, it is desirable to slow the absorption of the drug from subcutaneous or intramuscular injection. This may be accomplished by the use of a liquid suspension of crystalline or amorphous material having poor water solubility. The rate of absorption of the drug then depends upon its rate of dissolution which, in turn, may depend

upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally-administered drug form is accomplished by dissolving or suspending the drug in an oil vehicle.

Injectable depot forms are made by forming microencapsule matrices of vitamin D compound(s) in biodegradable polymers such as polylactide-polyglycolide. Depending on the ratio of drug to polymer, and the nature of the particular polymer employed, the rate of drug release can be controlled. Examples of other biodegradable polymers include poly(orthoesters) and poly(anhydrides). Depot injectable formulations are also prepared by entrapping the drug in liposomes or microemulsions which are compatible with body tissue.

When the vitamin D compound(s) are administered as pharmaceuticals, to humans and animals, they can be given per se or as a pharmaceutical composition containing, for example, 0.1 to 99.5% (more preferably, 0.5 to 90%) of active ingredient in combination with a pharmaceutically-acceptable carrier.

Regardless of the route of administration selected, the vitamin D compound(s), which may be used in a suitable hydrated form, and/or the pharmaceutical compositions of the present invention, are formulated into pharmaceutically-acceptable dosage forms by conventional methods known to those of skill in the art.

Actual dosage levels and time course of administration of the active ingredients in the pharmaceutical compositions of the invention may be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient. An exemplary dose range is from 0.1 to 300 μg per day

A preferred dose of the vitamin D compound for the present invention is the maximum that a patient can tolerate and not develop hypercalcemia. Preferably, the vitamin D compound of the present invention is administered at

a concentration of about 0.001 μg to about 100 μg per kilogram of body weight, about 0.001 – about 10 μg/kg or about 0.001 μg – about 100 μg/kg of body weight. Ranges intermediate to the above-recited values are also intended to be part of the invention.

Synthesis of Compounds of the Invention

A number of the compounds of the present invention can be prepared by incubation of vitamin D_3 analogues in cells, for example, incubation of vitamin D_3 analogues in either UMR 106 cells or Ros 17/2.8 cells results in production of vitamin D_3 compounds of the invention. For example, Incubation of 1,25-dihydroxy-16-ene-5,6-trans-calcitriol in UMR 106 cells results in production of the 1,25-dihydroxy-16-ene-24-oxo-5,6-trans-calcitriol.

In addition to the methods described herein, compounds of the present invention can be prepared using a variety of synthetic methods. For example, one skilled in the art would be able to use methods for synthesizing existing vitamin D_3 compounds to prepare compounds of the invention (*see e.g.,* Bouillon, R. *et al.*, *Endocrine Reviews* 16(2):201-204; Ikekawa N. (1987) *Med. Res. Rev.* 7:333-366; DeLuca H.F. and Ostrem V.K. (1988) *Prog. Clin. Biol. Res.* 259:41-55; Ikekawa N. and Ishizuka S. (1992) *CRC Press* 8:293-316; Calverley M.J. and Jones G. (1992) *Academic Press* 193-270; Pardo R. and Santelli M. (1985) *Bull. Soc. Chim.* Fr:98-114; Bythgoe B. (1980) *Chem. Soc. Rev.* 449-475; Quinkert G. (1985) *Synform* 3:41-122; Quinkert G. (1986) *Synform* 4:131-256; Quinkert G. (1987) *Synform* 5:1-85; Mathieu C. *et al.* (1994) *Diabetologia* 37:552-558; Dai H. and Posner G.H. (1994) *Synthesis* 1383-1398); DeLuca *et al.*, WO 97/11053.

Exemplary methods of synthesis include the photochemical ring opening of a 1-hydroxylated side chain-modified derivative of 7-dehydrocholesterol which initially produces a previtamin that is easily thermolyzed to vitamin D_3 in a well known fashion (Barton D.H.R. *et al.* (1973) *J. Am. Chem. Soc.* 95:2748-2749; Barton D.H.R. (1974) *JCS Chem. Comm.*

203-204); phosphine oxide coupling method developed by (Lythgoe et al (1978) *JCS Perkin Trans.* 1:590-595) which comprises coupling a phosphine oxide to a Grundmann's ketone derivative to directly produce a 1α,25$(OH)_2D_3$ skeleton as described in Baggiolini *E.G., et al.* (1986) *J. Org. Chem.* 51:3098-3108; DeSchrijver J. and DeClercq P.J. (1993) *Tetrahed Lett* 34:4369-4372; Posner G.H and Kinter C.M. (1990) *J. Org. Chem.* 55:3967-3969; semihydrogenation of dienynes to a previtamin structure that undergoes rearrangement to the corresponding vitamin D_3 analogue as described by Harrison R.G. *et al.* (1974) *JCS Perkin Trans.* 1:2654-2657; Castedo L. *et al.* (1988) *Tetrahed Lett* 29:1203-1206; Mascarenas J.S. (1991) *Tetrahedron* 47:3485-3498; Barrack S.A. *et al.* (1988) *J. Org. Chem.* 53:1790-1796) and Okamura W.H. *et al.* (1989) *J. Org. Chem.* 54:4072-4083; the vinylallene approach involving intermediates that are subsequently arranged using heat or a combination of metal catalyzed isomerization followed by sensitized photoisomerization (Okamura W.H. *et al.* (1989) *J. Org. Chem.* 54:4072-4083; Van Alstyne E.M. *et al.* (1994) *J. Am. Chem. Soc.*116:6207-6210); the method described by Trost *et al.* B.M. *et al. J. Am. Chem. Soc.* 114:9836-9845; Nagasawa K. *et al.* (1991) *Tetrahed Lett* 32:4937-4940 involves an acyclic A-ring precursor which is intramolecular cross-coupled to the bromoenyne leading directly to the formation of 1,25$(OH)_2D_3$ skeleton; a tosylated derivative which is isomerized to the i-steroid that can be modified at carbon-1 and then subsequently back-isomerized under sovolytic conditions to form 1α ,25$(OH)_2D_2$ or analogues thereof (Sheves M. and Mazur Y. (1974) *J. Am. Chem. Soc.* 97:6249-6250; Paaren H.E. *et al.* (1980) *J. Org. Chem.* 45:3253-3258; Kabat M. *et al.* (1991) *Tetrahed Lett* 32:2343-2346; Wilson S.R. *et al.* (1991) *Tetrahed Lett* 32:2339-2342); the direct modification of vitamin D derivatives to 1-oxygenated 5, 6-trans vitamin D as described in (Andrews D.R. *et al.* (1986) *J. Org. Chem.* 51:1635-1637); the Diels-Alders cycloadduct method of previtamin D_3 can be used to cyclorevert to 1α,25$(OH)_2D_2$ through

the intermediary of a previtamin form via thermal isomerization (Vanmaele L. *et al.* (1985) *Tetrahedron* 41:141-144); and, a final method entails the direct modification of 1α,25$(OH)_2D_2$ or an analogue through use of suitable protecting groups such as transition metal derivatives or by other chemical transformations (Okarmura W.H. *et al.* (1992) *J. Cell Biochem.* 49:10-18). Additional methods for synthesizing vitamins D2 compounds are described in, for example, Japanese Patent Disclosures Nos. 62750/73, 26858/76, 26859/76, and 71456/77; U.S. Pat. Nos. 3,639,596; 3,715,374; 3,847,955 and 3,739,001.

Examples of the compounds of this invention having a saturated side chain can be prepared according to the general process illustrated and described in U.S. Patent No. 4,927,815. Examples of compounds of the invention having an unsaturated side chain can be prepared according to the general process illustrated and described in U.S. Patent No. 4,847,012. Examples of compounds of the invention wherein R groups together represent a cycloalkyl group can be prepared according to the general process illustrated and described in U.S. Patent No. 4,851,401.

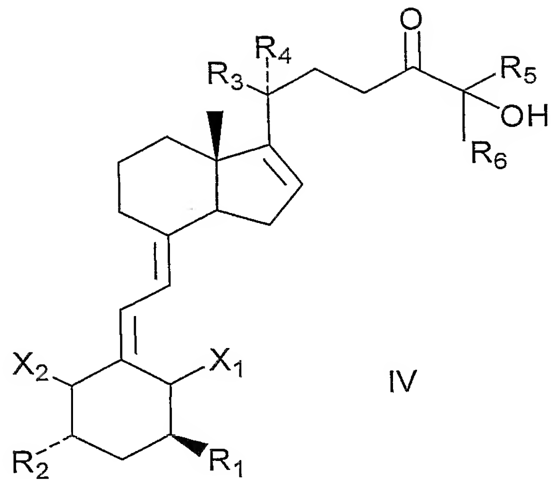
Another synthetic strategy for the preparation of side-chain-modified analogues of 1α,25-dihydroxyergocalciferol is disclosed in Kutner *et al.*, *The Journal of Organic Chemistry*, 1988, 53:3450-3457. In addition, the preparation of 24-homo and 26-homo vitamin D analogues are disclosed in U.S. Patent No. 4,717,721.

The enantioselective synthesis of chiral molecules is now state of the art. Through combinations of enantioselective synthesis and purification techniques, many chiral molecules can be synthesized as an enantiomerically enriched preparation. For example, methods have been reported for the enantioselective synthesis of A-ring diastereomers of 1α,25$(OH)_2D_3$ as described in Muralidharan *et al.* (1993) *J. Organic Chem.* 58(7): 1895-1899 and Norman *et al.* (1993) *J. Biol. Chem.* 268(27): 20022-30. Other methods

for the enantiomeric synthesis of various compounds known in the art include, *inter alia*, epoxides (see, *e.g.,* Johnson, R.A.; Sharpless, K.B. In *Catalytic Asymmetric Synthesis*; Ojima, I., Ed.: VCH: New York, 1993; Chapter 4.1. Jacobsen, E.N. *Ibid.* Chapter 4.2), diols (*e.g.,* by the method of Sharpless, *J. Org. Chem.* (1992) 57:2768), and alcohols (*e.g.,* by reduction of ketones, E.J.Corey *et al.*, *J. Am. Chem. Soc.* (1987) 109:5551). Other reactions useful for generating optically enriched products include hydrogenation of olefins (*e.g.,* M. Kitamura *et al.*, *J. Org. Chem.* (1988) 53:708); Diels-Alder reactions (*e.g.,* K. Narasaka *et al.*, *J. Am. Chem. Soc.* (1989) 111:5340); aldol reactions and alkylation of enolates (see, *e.g.,* D.A. Evans *et al.*, *J. Am. Chem. Soc.* (1981) 103:2127; D.A. Evans *et al.*, *J. Am. Chem. Soc.* (1982) 104:1737); carbonyl additions (*e.g.,* R. Noyori, *Angew. Chem. Int. Ed. Eng.* (1991) 30:49); and ring-opening of meso-epoxides (*e.g.,* Martinez, L.E.; Leighton J.L., Carsten, D.H.; Jacobsen, E.N. *J. Am. Chem. Soc.* (1995) 117:5897-5898). The use of enzymes to produce optically enriched products is also well known in the art (*e.g.,* M.P. Scheider, ed. "Enzymes as Catalysts in Organic Synthesis", D. Reidel, Dordrecht (1986).

Chiral synthesis can result in products of high stereoisomer purity. However, in some cases, the stereoisomer purity of the product is not sufficiently high. The skilled artisan will appreciate that the separation methods described herein can be used to further enhance the stereoisomer purity of the vitamin D_3-epimer obtained by chiral synthesis.

Compounds of formula (IV)



IV

wherein:

X_1 and X_1 are each independently H_2 or $=CH_2$, provided X_1 and X_1 are not both $=CH_2$;

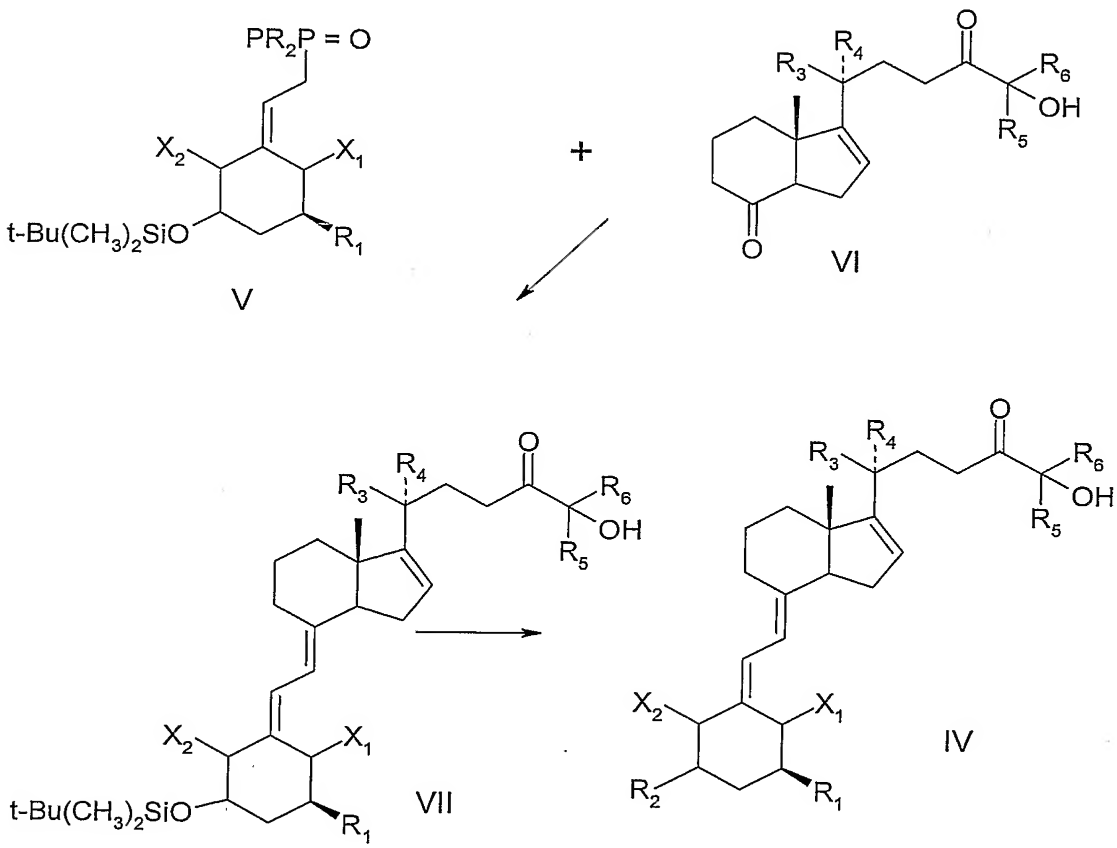
R_1 and R_2 are each independently, hydroxyl, OC(O)C_1-C_4 alkyl, OC(O)hydroxyalkyl, OC(O)fluroralkyl, provided that R_1 and R_2 are not both hydroxyl;

R_3 and R_4 are each independently hydrogen, C_1-C_4 alkyl, or R_3 and R_4 taken together with C_{20} form C_3-C_6 cycloalkyl; and

R_5 and R_6 are each independently C_1-C_4 alkyl, hydroxyalkyl, or haloalkyl, *e.g.*, fluoroalkyl, *e.g.*, fluoromethyl and trifluoromethyl; and pharmaceutically acceptable esters, salts, and prodrugs thereof, can be synthesized by methods described in this section, and the chemical literature In particular, compounds of formula IV of the invention are prepared as shown in Scheme 1 below. Accordingly, compounds of formula IV are prepared by coupling compounds of formula V with compounds of formula VI in tetrahydrofuran with n-butyllithium as a base to give compounds of formula VIII. Subsequent removal of the protecting silyl groups (R_1 = $OSi(CH_3)_2$t.Bu) affords the 1,3

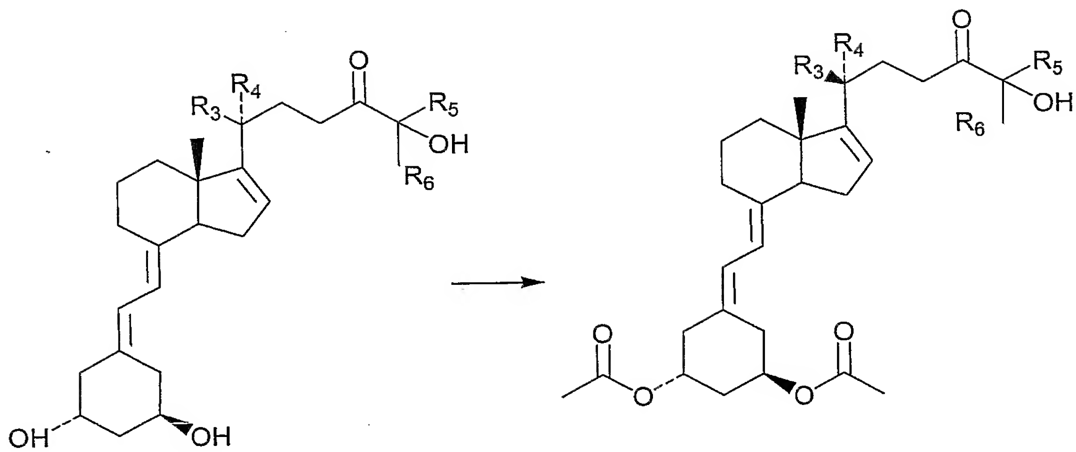
dihydroxy vitamin D_3 compound of formula IV (R_1 = OH, R_2 = OH). Acylation at the 1 and/or 3 positions is achieved using methods well-known in the art. For example, preparation of the 1,3 diacetoxy compounds of formula IV (R_1 = R_2 = OAc) requires additional acetylation with acetic anhydride and pyridine, as shown in Scheme 2 and described below.

Scheme 1



wherein X_1, X_2, R_3, R_4, R_5 and R_6 are as defined above.

Scheme 2

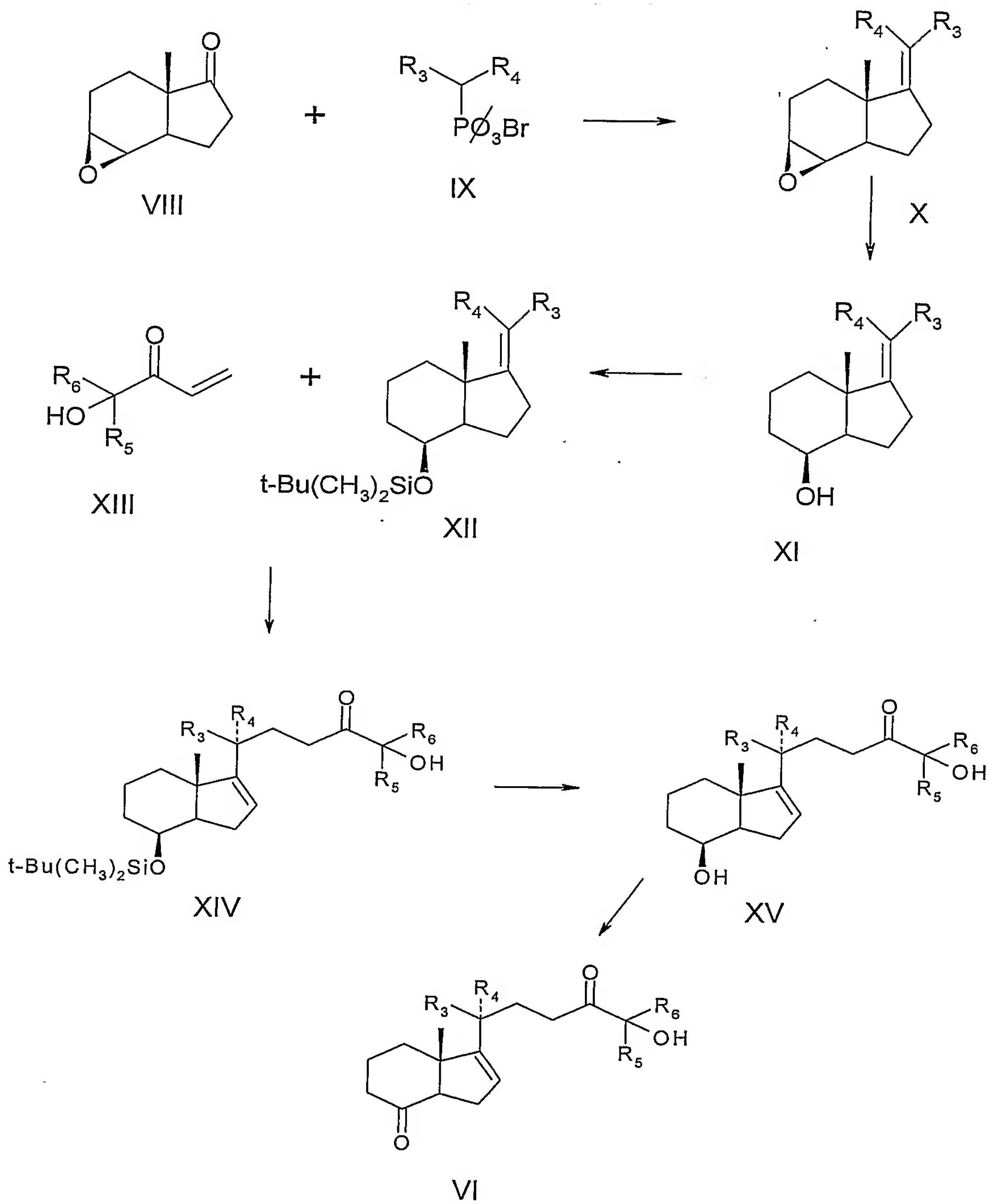


1,25-dihydroxy-16-ene-24-keto-19-nor-cholecalciferol
(1)

1,3-O-diacetyl-1,25-dihydroxy-16-ene-24-keto-19-nor-cholecalciferol
(2)

Referring to Schemes 1 and 3, compounds of formula V are known compounds, and are prepared starting from the known epoxy-ketone of formula VIII. The compound of formula VIII is converted to the epoxy-olefin of formula X by a Wittig reaction. Reduction with $LiAlH_4$ to the compound XI and protection of the hydroxy group resulted in compound XII. Then, the ene reaction of forumula XII with the known hydroxy-conjugated ketone XIII (R_5 = R_6 = CH_3) in tetrahydrofuran, in the presence of Lewis acid $(CH_3)_2$ Al Cl, provides the compound XIV featuring the C,D-rings and full side chain of the target vitamin D analogs. Finally, removal of the silyl group and oxidation provides the key intermediate, Ketone of formula VI.

Scheme 3

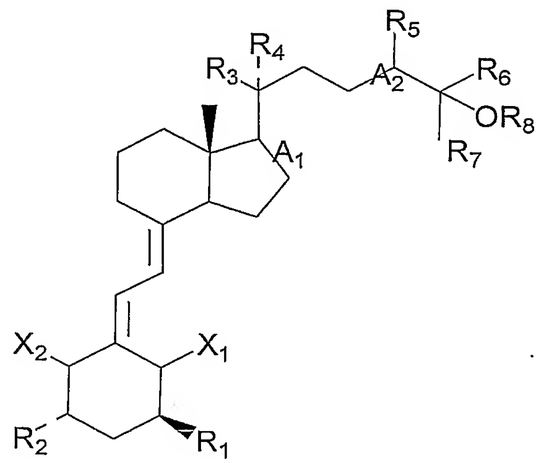


Referring to Scheme 2, synthesis of 1,3-O-Diacetyl-1,25-Dihydroxy-16-ene-24-Keto-19-nor-Cholecalciferol (2) may be carried out as follows:

0.032 g of 1,25-dihydroxy-16-ene-24-keto-19-nor-cholecalciferol **(1)** was dissolved in 0.8 ml pyridine, cooled in bath and treated with 0.2 ml acetic anhydride for 7 hours at room temperature and for 14 hours in a refrigerator. It was then diluted with 1 ml of water, stirred for 10 min in an ice bath, diluted with 5 ml water and 20 ml ethyl acetate. The organic layer was washed with 3 x 5 ml of water, then with 5 ml saturated sodium bicarbonate, then with brine, dried over sodium sulfate and evaporated. The oily residue was taken up in 1:6 ethyl acetate-hexane, then flash chromatographed on a 13.5 x 110 mm column using 1:6 ethyl acetate-hexane as mobile phase for fractions 1 – 5, 1:4 ethyl acetate-hexane for the remaining fractions. Fractions 11 – 14 were pooled and evaporated to give 0.0184 g of the title compound **(2)**.

In this and the following synthesis example (synthesis of 1,3-Di-O-acetyl-1,25-dihydroxy-16,23Z-diene-26,27-hexafluoro-19-nor-cholecalciferol) all operations involving vitamin D_3 analogs were conducted in amber-colored glassware in a nitrogen atmosphere. Tetrahydrofuran was distilled from sodium-benzophenone ketyl just prior to its use and solutions of solutes were dried with sodium sulfate. Melting points were determined on a Thomas-Hoover capillary apparatus and are uncorrected. Optical rotations were measured at 25 °C. ^1H NMR spectra were recorded at 400 MHz in $CDCl_3$ unless indicated otherwise. TLC was carried out on silica gel plates (Merck PF-254) with visualization under short-wavelength UV light or by spraying the plates with 10% phosphomolybdic acid in methanol followed by heating. Flash chromatography was carried out on 40-65 μm mesh silica gel. Preparative HPLC was performed on a 5×50 cm column and 15-30 μm mesh silica gel at a flow rate of 100 ml/min.

Vitamin D_3 compounds of the formula:



wherein:

A_1 is single or double bond;

A_2 is a single, double or triple bond;

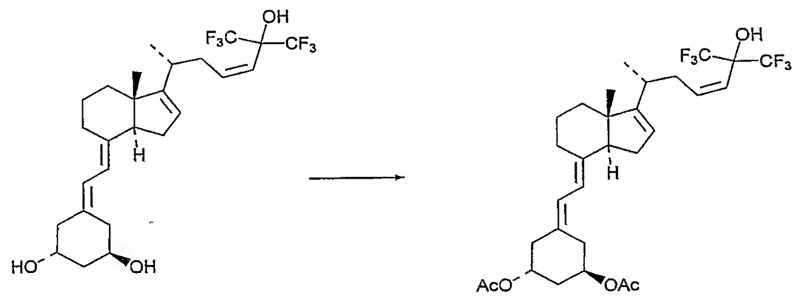
X_1 and X_2 are each independently H or $=CH_2$,

R_1 and R_2 are each independently OC(O)C1-C4 alkyl, OC(O)hydroxyalkyl, or OROC(O)haloalkyl;

R_3, R_4 and R_5 are each independently hydrogen, C1-C4 alkyl, hydroxyalkyl, or haloalkyl, or R_3 and R_4 taken together with C20 form C3-C6 cycloalkyl;

R_6 and R_7 are each independently haloalkyl; and

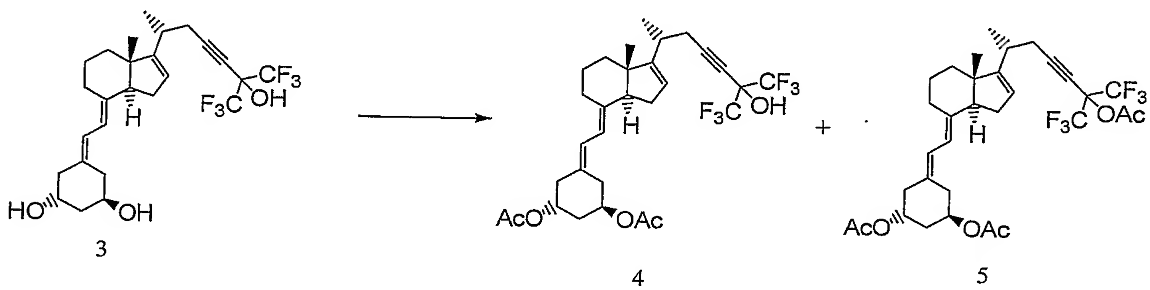
R_8 is H or OC(O)C1-C4 alkyl, OC(O)hydroxyalkyl, or OROC(O)haloalkyl; and pharmaceutically acceptable esters, salts, and prodrugs thereof.may be prepared analogously to the synthesis of 1,3-Di-O-acetyl-1,25-dihydroxy-16,23Z-diene-26,27-hexafluoro-19-nor-cholecalciferol (2), which is carried out as follows:



1 2

The starting material 1,25-dihydroxy-16,23Z-diene-26,27-hexafluoro-19-nor-cholecalciferol **(1)** can be prepared as described in US Patent 5,428,029 to Doran et al.. 3 mg of 1,25-dihydroxy-16,23Z-diene-26,27-hexafluoro-19-nor-cholecalciferol **(1)** was dissolved in 0.8 ml of pyridine, cooled to ice-bath temperature and 0.2 ml of acetic anhydride was added and maintained at that temperature for 16 h. Then the reaction mixture was diluted with 1 ml of water, stirred for 10 min in the ice bath and distributed between 5 ml of water and 20 ml of ethyl acetate. The organic layer was washed with 3 x 5 ml of water, once with 5 ml of saturated sodium hydrogen carbonate, once with 3 ml of brine then dried (sodium sulfate) and evaporated. The oily residue was taken up in 1:6 ethyl acetate – hexane and flash-chromatographed using a stepwise gradient of 1:6, 1:4 and 1:2 ethyl acetate - hexane. The column chromatography was monitored by TLC (1:4 ethyl acetate – hexane, spot visualization with phosphomolybdic acid spray), the appropriate fractions were pooled, evaporated, the residue taken up in methyl formate, filtered, then evaporated again to give 23.8 mg of the title compound (**2**) as a colorless syrup; 400 MHz ^{1}H NMR δ 0.66 (3H, s), 0.90 (1H, m), 1.06 (3H, d, J=7.2 Hz), 1.51 (1H, m), 1.72-1.82 (3H,m), 1.9-2.1 (3H, m), 1.99 (3H, s) 2.04 (3H,s), 2.2-2.3 (3 m), 2.44-2.64 (6H, m), 2.78 (1H, m), 3.01 (1H, s), 5.10 (2H, m). 5.38 (1H, m), 5.43 (1H, d, J=12 Hz), 5.85 (1H, d, J=11.5 Hz), 5.97 (1H, dt, J=12 and 7.3 Hz), 6.25 (1H, d, J= 11.5 Hz).

Furthermore, synthesis of 1,3-Di-O-acetyl-1,25-Dihydroxy-16-ene-23-yne-26,27-hexafluoro-19-nor-cholecalciferol (4) and 1,3,25-Tri-O-acetyl-1,25-Dihydroxy-16-ene-23-yne-26,27-hexafluoro-19-nor-cholecalciferol (5) may be carried out as follows:



The starting material 1,25-dihydroxy-16-ene-23-yne-26,27-hexafluoro-19-nor-cholecalciferol **(3)** can be prepared as described in US Patents 5,451,574 and 5,612,328 to Baggiolini et al.. 314 mg (0.619 mmole) of 1,25-dihydroxy-16-ene-23-yne-26,27-hexafluoro-19-nor-cholecalciferol **(3)** was dissolved in 1.5 ml of pyridine, cooled to ice-bath temperature, and 0.4 ml of acetic anhydride was added. The reaction mixture was kept at room temperature for 7 hours and then for 23 hours in a refrigerator. It was then diluted with 10 ml water and extracted with 30 ml of ethyl acetate. The organic extract was washed with water and brine, dried over sodium sulfate and evaporated. The residue was FLASH chromatographed on a 10 x 140 mm column with 1:6 and 1:4 ethyl acetate-hexane as the mobile phase to give 126 mg of 1,3-Di-O-acetyl-1,25-Dihydroxy-16-ene-23-yne-26,27-hexafluoro-19-nor-cholecalciferol **(4)**, and 248 mg of 1,3,25-Tri-O-acetyl-1,25-Dihydroxy-16-ene-23-yne-26,27-hexafluoro-19-nor-cholecalciferol **(5)**.

The present invention will now be described with reference to the following non-limiting examples, with reference to the figures, in which:

Figure 1 shows the immunohistochemical detection of vitamin D receptors (VDRs) in bladder tissue.
Figure 2 shows the effect of calcitriol on bladder cell growth. "hB" = human bladder- "T" = testosterone; "C" = control.
Figure 3 shows the effect of a vitamin D compound on testosterone-stimulated bladder cell growth. "hB" = human bladder.
Figure 4 shows the effect of different compounds on stimulated and basal bladder cell growth. "T 10 nM" = testosterone; "F 1nM" = finasteride; "Cyp 100 nM" =cyproterone acetate.
Figure 5 shows the effect of a vitamin D compound on bladder weight.
Figure 6 shows the effect of a vitamin D compound on spontaneous non-voiding contraction frequency.
Figure 7 shows the effect of a vitamin D compound on spontaneous non-voiding contraction amplitude.
Figure 8 shows the effect of a vitamin D compound on micturition pressure.
Figure 9 shows the effect of a vitamin D compound on residual urine.
Figure 10 shows the effect of a vitamin D compound on the contractile response of bladder strips to EFS (Electrical Field Stimulation).
Figure 11 shows a comparison between cystometric parameters recorded in rats treated with a vitamin D3 analogue "Compound C" and control (vehicle treated) rats.

EXAMPLES

As described in the following examples, the biological hypothesis that Calcitriol and Vitamin D_3 analogues can have an effect on the growth and

function of bladder cells has been proven in *in vitro* models by culturing human stromal bladder cells and has been confirmed in a preclinical *in vivo* validated model .

Example 1: The activity of Calcitriol and Vitamin D_3 analogues on the growth and function of bladder cells

The biological hypothesis that Calcitriol and Vitamin D_3 analogues can have an effect on the growth and function of bladder cells has been proven in *in vitro* models by culturing human stromal bladder cells The Inventor confirmed the presence of vitamin D receptors (VDRs), as previously reported in the literature, on these cells (see below in Figure 1),.

In these models, Calcitriol (the activated form of vitamin D_3) and other vitamin D_3 analogues have been shown to be effective in inhibiting the basal and testosterone- stimulated growth of bladder cells. This activity, never reported before, is dose–dependent with a IC50 of $9.8 \pm 7x10^{-15}$ for Calcitriol (1,25 dihydroxycholecalciferol) (on basal cells) and of $1.6 \pm 7x10^{-15}$ for 1-alpha-fluoro-25-hydroxy-16,23e-diene-26,27-bishomo-20-epi-cholecalciferol ("Compound A"/"Cmpd A" in the figures) (on stimulated cells) (see Figure 2 and Figure 3).

This effect , demonstrated also with other vitamin D_3 analogues [e.g Ro 23-7553 (1,25-dihydroxy-16-ene-23-yne cholecalciferol) described in US Patent 5,145,846 and referred to as "Compound B"/"Cmpd B" in these Examples and the Figures] was, in some cases, significantly greater than that of anti-androgens widely used in the treatment of uro-genital diseases (Figure 4).

Example 2: The effect of vitamin D3 analogues on bladder dysfunction in a bladder outlet obstruction model

Experimental

1. Materials

1.1. Animals:

Female Sprague-Dawley rats, weighing 200-250g

1.2. Grouping

Group A: BOO rats, treated with the vitamin D analogue over 2 weeks, beginning at day 1 after creation of the obstruction (n=12)

Group B: BOO rats, treated with vehicle over 2 weeks, beginning at day 1 after creation of the obstruction (n=12)

Group C: Sham operated rats, treated with the vitamin D analogue over 2 weeks, beginning at day 1 after surgery (n=12)

1.3. Studies:

a) Cystometry (~ 18 hours after last administration of the drug/ vehicle, 12 hours after removal of the obstructing ligature) under conscious conditions.

b) Measurements of bladder weight

c) In vitro investigations

2. Methods

2.1. BOO:

The bladder and urethrovesical junction were exposed through a lower abdominal midline incision. A 0.9 mm metal rod was placed alongside the proximal urethra and a 3-0 silk ligature was tied tightly around the urethra and the rod, which was consequently be removed. Sham surgery was be performed accordingly, without placing the ligature. After 13 days the ligature

was be removed and a catheter was be inserted into the bladder dome and tunneled subcutaneously.

2.2. Cystometry

The following morning after insertion of the catheter, the cystometric investigation was performed without any anesthesia or restraint in a metabolic cage. The amount of voided urine was measured by means of a fluid collector, connected to a force displacement transducer. The bladder was continuously filled with saline at room temperature. The catheter was also connected to a pressure transducer. After a stabilization period of 30–60 minutes, when reproducible voiding patterns are achieved, the following parameters were recorded over a period of 30 minutes: Basal bladder pressure, micturition pressure, threshold pressure, micturition interval and volume, and non-voiding contractions. The amount of residual urine was investigated manually 3 times, at the end of the cystometry. Bladder capacity was calculated based on the measured values.

2.3. In vitro investigations

2.3.1.Preparations.

After completion of the cystometries, the rats were sacrified by carbon monoxide asphyxiation followed by exsanguination. The abdomen was accessed through a lower midline incision whereafter the symphysis was opened. The bladder was carefully dissected free, and immediately placed in chilled Krebs solution, and strip preparations were dissected.

2.3.2 Recording of mechanical activity.

The bladder and urethra were separated at the level of the bladder neck, and semicircular strips were prepared from the middle third of the detrusor (1 x 2 x 5 mm). All preparations were used immediately after removal.

The strips were transferred to 5 ml tissue baths containing Krebs solution. The Krebs solution was maintained at 37°C and bubbled continuously with a mixture of 95% O_2 and 5 % CO_2, resulting in a pH of 7.4. The strips were suspended between two L-shaped hooks by means of silk ligatures. One hook was connected to a movable unit allowing adjustment of passive tension, and the other to a Grass FT03C (Grass Instruments Co, MA, USA) force transducer. Isometric tension was recorded using a Grass polygraph (7D). After mounting, the strips were stretched to a passive tension of 4 mN (the same tension for all preparations) and allowed to equilibrate for 45-60 min before further experiments were performed.

2.3.3. Electrical field stimulation

Electrical field stimulation (EFS) was accomplished by means of two platinum electrodes placed on either side of the preparations, and was performed using a Grass S48 or S88 stimulator, delivering single square wave pulses at selected frequencies. The train duration was 5 s, the pulse duration 0.8 ms, and the stimulation interval 2 min. The polarity of the electrodes was shifted after each pulse by means of a polarity changing unit.

2.3.4 Procedure

Each experiment was started by exposing the preparations to a high K^+ (124 mM) Krebs solution until two reproducible contractions are obtained. Then the following experiments were carried out:

a) Electrical stimulation of nerves was performed and frequency-response relations obtained, in the presence and absence of atropine.

b) Concentration-response curves were constructed for carbachol and ATP

Results

The validated bladder outlet obstruction rat model described above was used to test the ability of vitamin D3 analogues to control and treat bladder

dysfunction.. The objective was to evaluate whether a vitamin D3 analogue (1-alpha-fluoro-25-hydroxy-16,23e-diene-26,27-bishomo-20-epi-cholecalciferol – *compound "A"*) at the dose of 150 µg/Kg/daily) can prevent bladder hypertrophy and bladder dysfunction such as bladder overactivity.

In this model a ligature was surgically placed around the outlet of the catheterized bladder, so that when the catheter was removed, the bladder experienced increased urethral resistance. The rats underwent continuous cystometry to evaluate bladder function. In addition the contractile properties of isolated bladder preparation in response to nerve stimulation and exogenous stimuli *in vitro* were investigated under electrical field stimulation (EFS).

The following cystometric parameters were investigated (see Figures 5-9):

micturition pressure (the maximum bladder pressure during micturition),

bladder capacity (residual volume after voiding plus the volume of saline infused to induce the void)

micturition volume (volume of the expelled urine)

residual urine (bladder capacity minus micturition volume)

and

frequency and amplitude of spontaneously occurring changes intravesical pressure (non-voiding contractions).

In this model the analogue under evaluation had a beneficial effect on bladder function . This effect was evident in the normal bladder and is maintained in bladder outlet obstruction. In particular significant differences versus vehicle were observed in:

-spontaneous non-voiding contraction frequency and amplitude (Figures 6 and 7);
-residual urine (absent with the active compound, Figure 9);
- micturition pressure (Figure 8);

In addition a beneficial effect on bladder function has been confirmed in the *in vitro* tests:
-K response;
-response to EFS (Figure 10);
-response to carbachol.

Finally a slight decrease in bladder weight was observed with the analogue tested (Figure 5).

These data demonstrate the use of vitamin D analogues (in the dose range from 50 µg to 300 µg - equivalent to approximately 0.725 to 5 µg/kg of body mass in humans) in the prevention and treatment of bladder dysfunction, such as overactive bladder. Without wishing to be bound by theory, the Inventor believes that the mechanism by which vitamin D analogues can be used to treat such diseases involves restricting abnormal proliferation of stromal and muscular cells of the bladder, which can lead to bladder dysfunction. However, the Inventors cannot exclude additional mechanisms of action for the compounds of the invention.

Example 3 Soft Gelatin Capsule Formulation I

Item	Ingredients	mg/Capsule
1	1-alpha-fluoro-25-hydroxy-16,23E-diene-26,27-bishomo-20-epi-cholecalciferol	10.001-0.02
2	Butylated Hydroxytoluene (BHT)	0.016
3	Butylated Hydroxyanisole (BHA)	0.016
4	Miglyol 812 qs.	160.0

Manufacturing Procedure:

1. BHT and BHA is suspended in Miglyol 812 and warmed to about 50 °C with stirring, until dissolved.
2. 1-alpha-fluoro-25-hydroxy-16,23E-diene-26,27-bishomo-20-epi-cholecalciferol is dissolved in the solution from step 1 at 50 °C.
3. The solution from Step 2 is cooled at room temperature.
4. The solution from Step 3 is filled into soft gelatin capsules.

Note: All manufacturing steps are performed under a nitrogen atmosphere and protected from light.

Example 4 Soft Gelatin Capsule Formulation II

Item	Ingredients	mg/Capsule
1	1-alpha-fluoro-25-hydroxy-16,23E-diene-26,27-bishomo-20-epi-cholecalciferol	10.001-0.02
2	di-.alpha.-Tocopherol	0.016
3	Miglyol 812 qs.	160.0

Manufacturing Procedure:

1. Di-α-Tocopherol is suspended in Miglyol 812 and warmed to about 50 °C with stirring, until dissolved.
2. 1-alpha-fluoro-25-hydroxy-16,23E-diene-26,27-bishomo-20-epi-cholecalciferol is dissolved in the solution from step 1 at 50 °C.
3. The solution from Step 2 is cooled at room temperature.
4. The solution from Step 3 is filled into soft gelatin capsules.

Example 5: Evaluation of the effect of Vitamin D3 analogues on bladder function in an *in vivo* model –cyclophosphamide (CYP) induced chronic IC in rats.

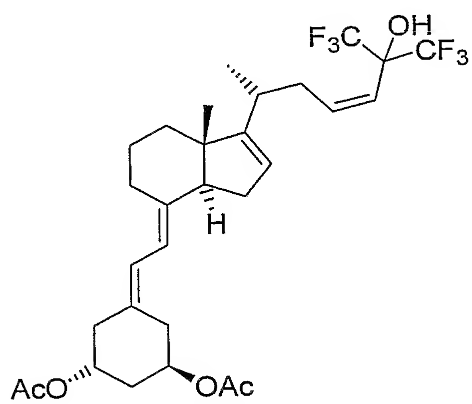
The rat model of chemical cystitis induced by intraperitoneal injection of CYP has been well accepted. CYP is used in clinical practice in the treatment of a number of malignant tumors. One of its metabolites, acrolein, is excreted in urine in large concentrations causing hemorrhagic cystitis associated with symptoms of urinary frequency, urgency and pelvic pain. The inflammatory process is characterized by changes in gross histology of bladder, increase in number and distribution of inflammatory cell infiltrates (mast cells, macrophage, PMNs), cyclo-oxygenase-2 expression and prostaglandin production, growth factor and cytokine production. The rat model of chemical cystitis closely resembles interstitial cystitis, a chronic, painful urinary bladder syndrome and has been used for the testing of therapeutic agents in the past.

This model was used to test the effects of 1,25-dihydroxyvitamin D3 analogue in rats with CYP-induced cystitis. The effects of the treatment on the cystometric parameters in a conscious freely moving rat with CYP-induced cystitis were monitored. The following cystometric parameters were recorded in each animal:

- bladder capacity
- filling pressure (pressure at the beginning of the bladder filling)
- threshold pressure (bladder pressure immediately prior to micturition)
- micturition pressure (the maximal bladder pressure during micturition)
- presence or absence of non-voidng bladder contractions (increases in bladder pressure of at least 10 cm H_20 without release of urine)
- amplitude of non-voididng bladder contraction.

Animals: Wistar rats weighing 125-175g were used. Two groups of animals had a tube implanted into the urinary bladder for intravesical pressure recording. Following recovery all animals received three intraperitoneal injections of CYP and subsequently were divided into the treatment and sham control groups.

Treatment group: Rats treated with oral 1,25-dihydroxyvitamin D3 analogue 1,3-Di-O-acetyl-1,25-dihydroxy-16,23Z-diene-26,27-hexafluoro-19-nor-cholecalciferol "Compound C" for 14 days (daily dose of 0.1 μg/kg)



"Compound C"

Control group: Rats treated with oral vehiculum (miglyol) in the dose identical to that delivered in the treatment group

Cystometry was performed 24 hours following the last dose of the drug or vehiculum on awake freely moving animals.

Number of animals per group:

Sham control animals	4
Treated animals	3

Methods

Implantation of the polyethylene tubing into the urinary bladder:

A lower midline abdominal incision was performed under general inhalation anesthesia (isoflurine with O2) and polyethylene tubing (PE-50, Clay Adams, Parsippany, NJ) with the end flared by heat was inserted into the dome of the bladder and secured in place with a 6-0 prolene purse string suture. The distal end of the tubing was heat-sealed, tunneled subcutaneously and externalized at the back of the neck, out of the animal's reach. Abdominal and neck incisions were closed with 4-0 nylon sutures.

Intraperitoneal injection of cyclophosphamide:

Following recovery (5 days) subject animals underwent three intraperitoneal injections of CYP (Sigma Chemical, St. Luis, MO; 75 mg/kg each, intraperitoneal) over the period of nine days. On the tenth day following the first CYP injection the sham control animals received the vehicle only, whereas the experimental group were treated with the 1,25-dihydroxyvitamin D3 analogue 1,3-Di-O-acetyl-1,25-dihydroxy-16,23Z-diene-26,27-hexafluoro-19-nor-cholecalciferol “Compound C” (delivered using gavage). Two weeks following the initiation of the treatment animals underwent a conscious cystometrogram to assess the function of the urinary bladder.

Cystometrogram

An animal was placed unrestrained in a cage and the catheter was connected via a T-tube to a pressure transducer (Grass® Model PT300, West Warwick, RI) and microinjection pump (Harvard Apparatus 22, South Natick, MA). A 0.9% saline solution was infused at room temperature into the bladder at a rate of 10 ml/hour. Intravesical pressure was recorded continuously using a Neurodata Acquisition System (Grass® Model 15, Astro-Med, Inc, West

Warwick, RI). At least three reproducible micturition cycles were recorded after the initial stabilization period of 25 - 30 minutes.

Timeline of an experiment:

Procedure	**Days**
Acclimation period	1 – 5
Tube implantation + recovery period	6 – 10
CYP treatment (three doses of 75mg/kg i.p. every three days)	11 – 17
Treatment (sham or active)	18 – 31
Cystometric evaluation	32

Results

The data analysis is summarized in Tables 1 and 2 and Figure 11 in which:

Bl. Cap = bladder capacity (ml)

FP = filling pressure (cmH_2O)

TP = threshold pressure (cmH_2O)

MP = micturition pressure (cmH_2O)

# of NVBC = number of non-voiding bladder contractions

amplitude of NVBC = amplitude of non-voiding bladder contraction

Rat	Bl. Cap.	FP	TP	MP	# of NVBC	Amplitude of NVBC
RB 8	1,2	15	15	100	22	15
	1,2	13	18	100	14	14
	1,1	16	15	82	12	11
RB10	0,7	30	40	110	26	25
	0,9	32	26	94	32	28
	0,6	26	26	108	35	16
RB12	1,7	35	40	115	40	17
	1,7	25	30	125	35	14
	1,9	30	25	118	22	17
RB14	1,3	16	16	104	10	10
	1,2	17	17	95	4	8
	1,1	19	21	92	9	18

Table 1: cystometric parameters for the control group.

Rat	Bl. Cap.	FP	TP	MP	# of NVBC	amplitude of NVBC
RB7	0,7	13	14	98	0	0
	0,7	14	14	97	0	0
	0,8	13	14	101	0	0
RB13	1,4	14	15	104	8	11
	1,9	15	16	105	4	10
	1,3	14	17	97	8	11
RB15	2,5	12	14	90	0	0
	1,3	11	12	100	0	0
	1,5	10	11	108	0	0

Table 2:cystometric parameters for the treatment group

Changes were noted in a number of cystometric parameters. Dramatic reductions in both the number and amplitude of non-voiding bladder contractions were observed in the drug treated animals. Less pronounced but still statistically significant reductions in the filling and threshold pressures were also recorded. The treatment did not result in a change of the bladder capacity.

Bladder overactivity associated with chronic cystitis manifests itself in frequent contractions of the bladder wall associated with irritative often painful urinary symptoms. The fact that non-voiding bladder contractions were

reduced both in their frequency and amplitude strongly suggest that if the effects on the bladder function in patients with interstitial cystitis will be similar, oral treatment with vitamin D3 analogues has a potential to relieve these debilitating symptoms. Reduction in filling and threshold pressures is significant from a clinical standpoint because the increased intravesical pressure associated with interstitial cystitis is a condition potentially jeopardizing the upper urinary tract.

This example provides a further demonstration that a vitamin D3 analogue, 1,3-Di-O-acetyl-1,25-dihydroxy-16,23Z-diene-26,27-hexafluoro-19-nor-cholecalciferol, has the ability to treat bladder dysfunction.

CLAIMS

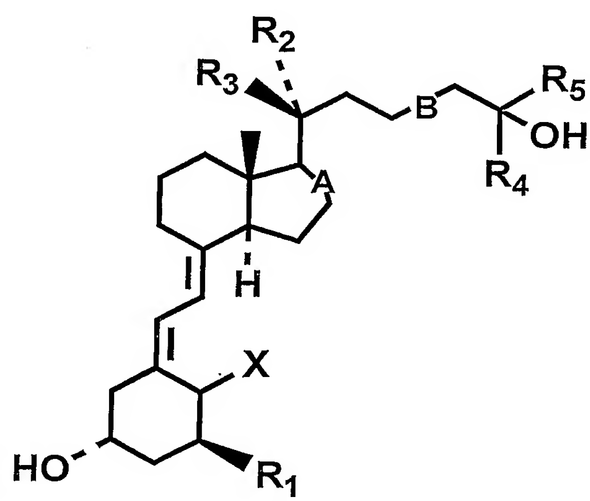
1. Use of a Vitamin D compound in the prevention or treatment of bladder dysfunction.

2. A method of treating a patient with bladder dysfunction by administering a effective amount of a Vitamin D compound.

3. The use of a Vitamin D compound as defined in claim 1 in the manufacture of a medicament for the prevention or treatment of bladder dysfunction.

4. The use or method of any one of claims 1 to 3, wherein said bladder dysfunction is characterized by the presence of bladder hypertrophy.

5. The use or method of any one of claims 1 to 4, wherein said vitamin D compound is a compound of the formula



X is H_2 or CH_2

R_1 is hydrogen, hydroxy or fluorine

R_2 is hydrogen or methyl

R_3 is hydrogen or methyl. When R_2 or R_3 is methyl, R_3 or R_2 must be hydrogen.

R_4 is methyl, ethyl or trifluoromethyl

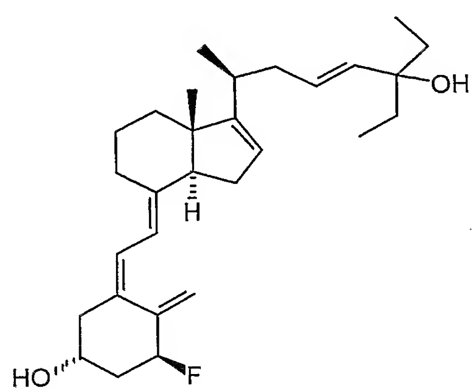
R_5 is methyl, ethyl or trifluoromethyl

A is a single or double bond

B is a single, E-double, Z-double or triple bond

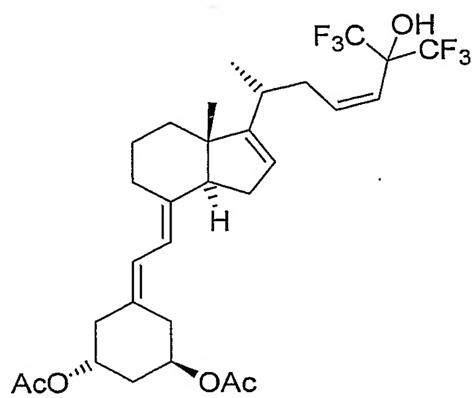
6. The use or method of any one of claims 1 to 4, wherein said vitamin D compound is a compound as defined in claim 5, wherein each of R_4 and R_5 is methyl or ethyl.

7. The use or method of claim 6 wherein said compound is 1-alpha-fluoro-25-hydroxy-16,23E-diene-26,27-bishomo-20-epi-cholecalciferol, having the formula:



8. The use or method of any one of claims 1 to 4 wherein said compound is 1,25-dihydroxy-16-ene-23-yne cholecalciferol.

9. The use or method of any one of claims 1 to 4, wherein said vitamin D compound is 1,3-Di-O-acetyl-1,25-dihydroxy-16,23Z-diene-26,27-hexafluoro-19-nor-cholecalciferol, having the formula:



FIGURES

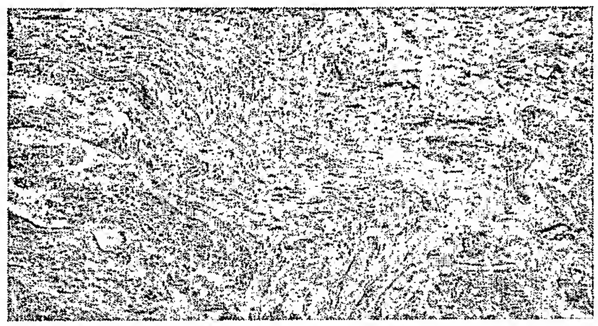


Figure 1:

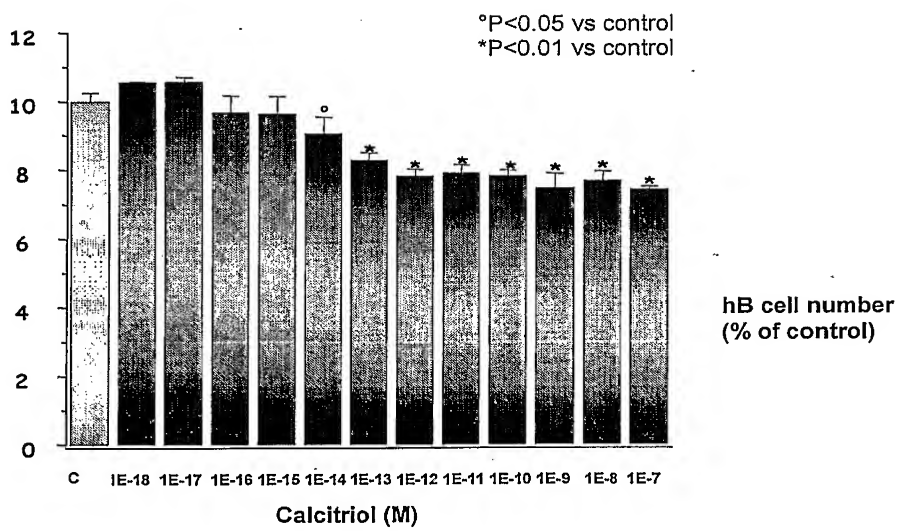


Figure 2.

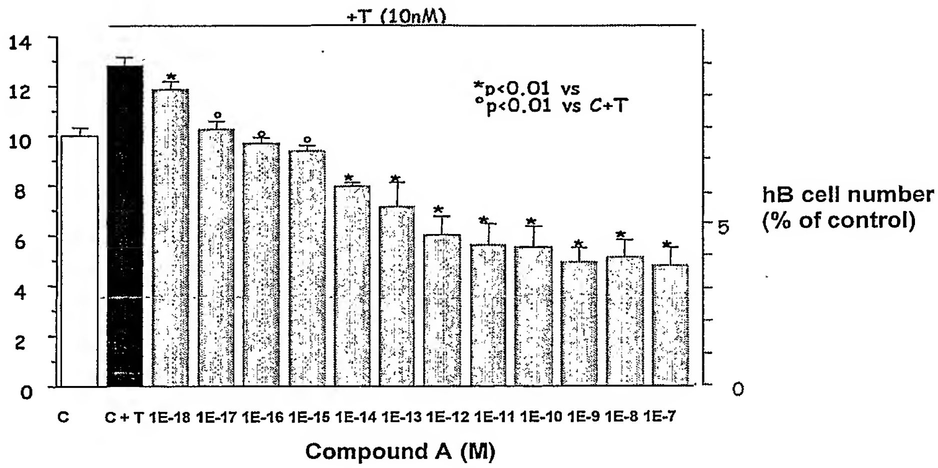


Figure 3:

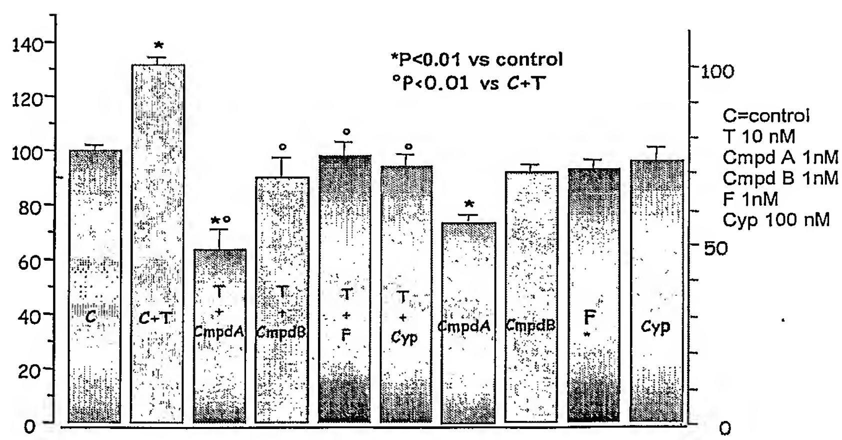


Figure 4:

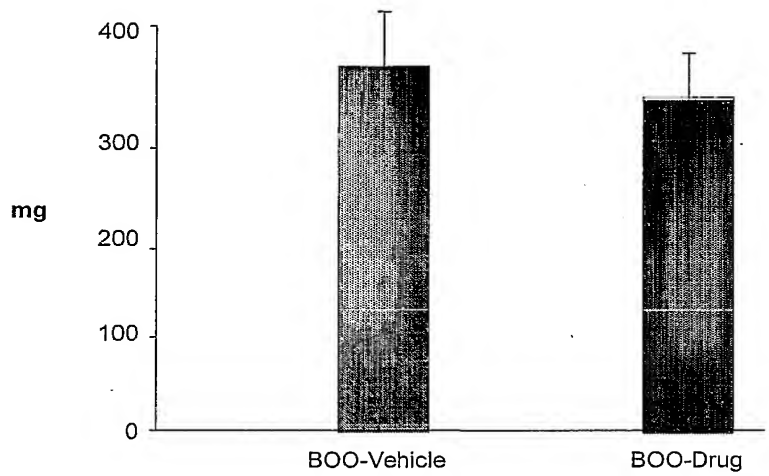


Figure 5:

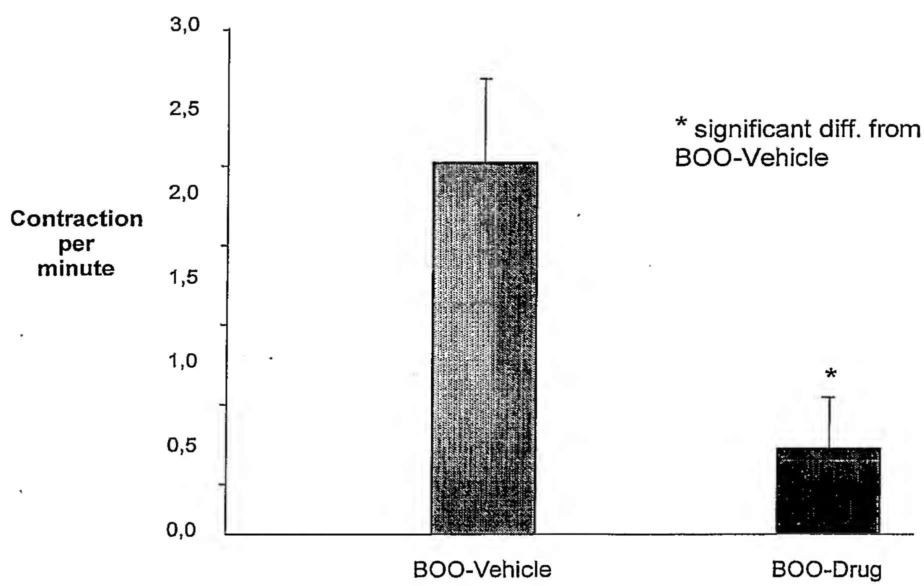


Figure 6:

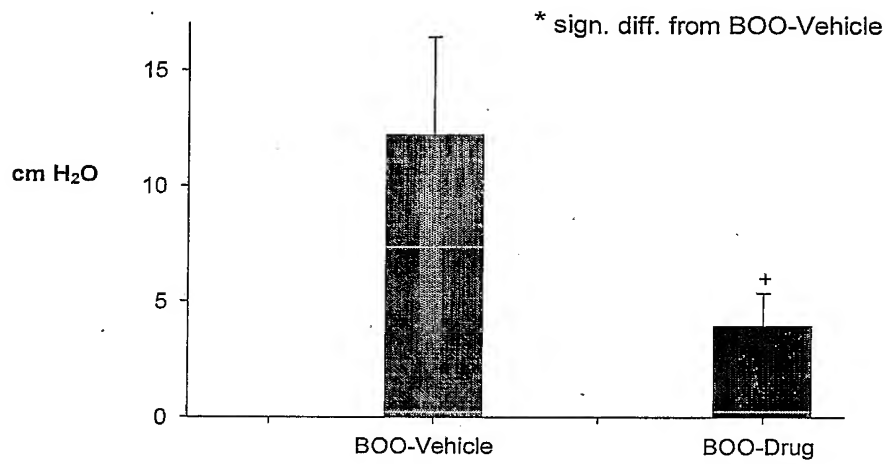


Figure 7.

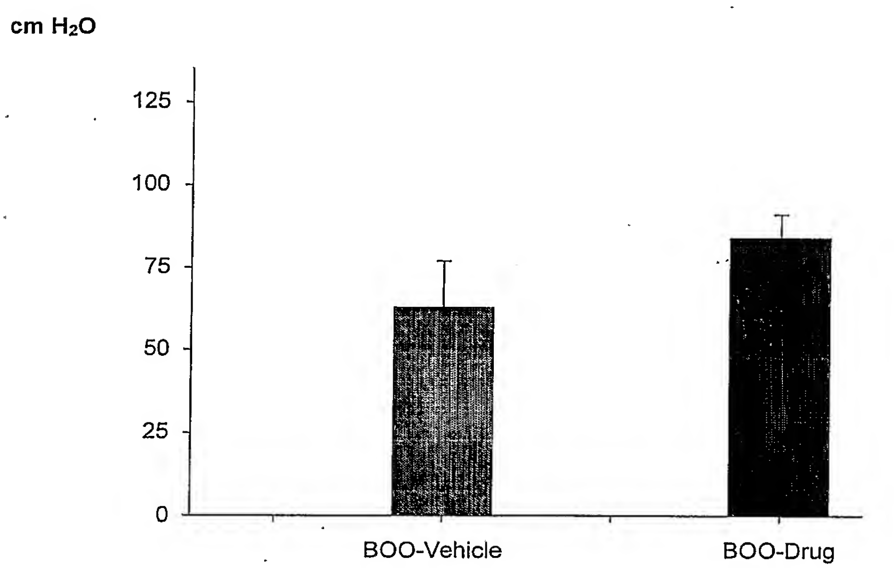


Figure 8.

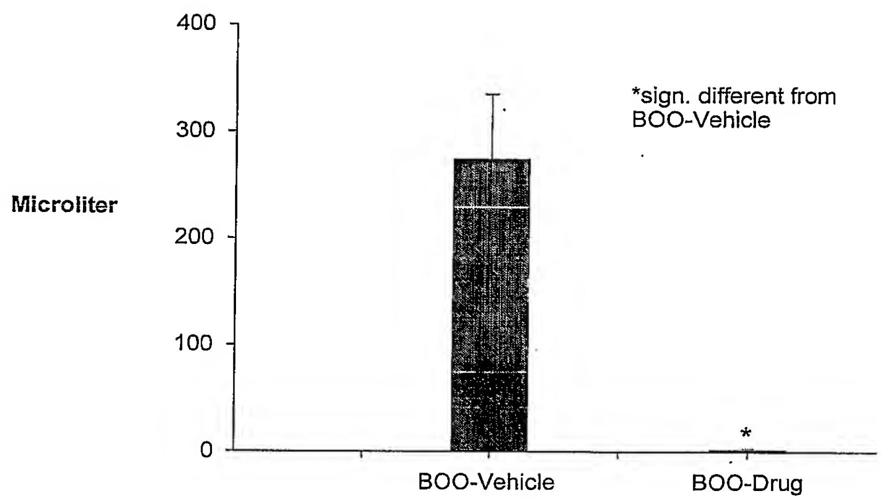

400
300
200
100
0
Microliter
*sign. different from
BOO-Vehicle
*
BOO-Vehicle
BOO-Drug

Figure 9

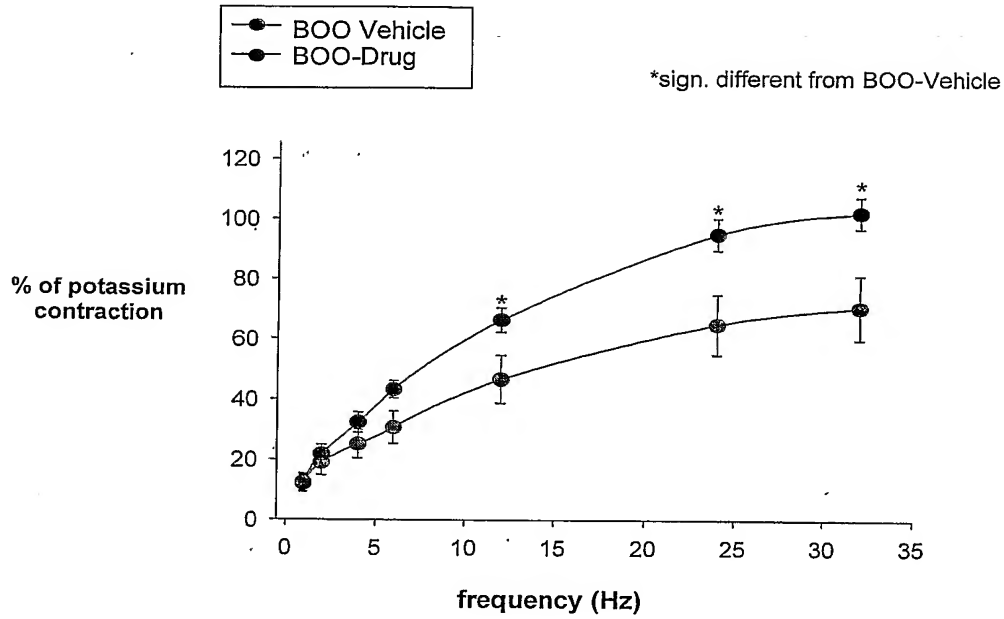

BOO Vehicle
BOO-Drug
*sign. different from BOO-Vehicle
% of potassium contraction
120
100
80
60
40
20
0
0
5
10
15
20
25
30
35
frequency (Hz)
*

Figure 10.

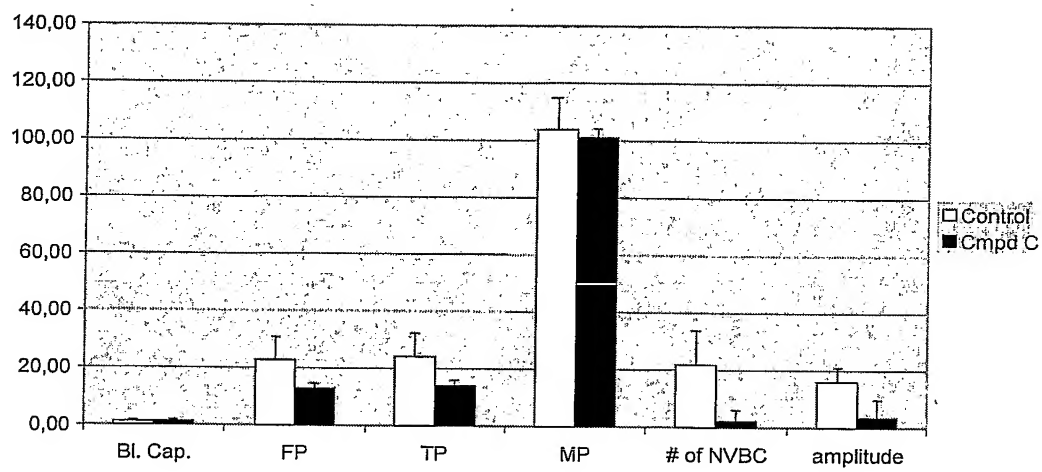

140,00
120,00
100,00
80,00
60,00
40,00
20,00
0,00
Bl. Cap.
FP
TP
MP
# of NVBC
amplitude
Control
Cmpd C

Figure 11.